
THE UNIVERSITY
*of* EDINBURGH

# A Critical Assessment Of Computed Tomography As A Valid Means Of Muscular Body Composition Analysis In Cancer Cachexia

Michael Ramage

Doctor of Medicine University of Edinburgh

2020

# Contents

# List of Tables

# List of Figures

# Declaration

I declare that the thesis has been composed by me and that the work has not be submitted for any other degree or professional qualification. I confirm that the work submitted is my own, except where work which has formed part of jointly-authored publications has been included. My contribution and those of the other authors to this work have been explicitly indicated below. I confirm that appropriate credit has been given within this thesis where reference has been made to the work of others.

The work presented in Chapter 1 was previously published in *Current Opinions in Palliative Care* as *"The relationship between muscle mass andfunction in cancer cachexia: smoke and mirrors?"* by *M. Ramage (Student)* and *R. Skipworth (Supervisor)*. This study was conceived by all of the authors. I carried out planning, literature search, composition, and editing of the work.

The work presented in Chapter 7 was previously published in *Clinical Nutrition* as *"The relationship between muscle protein content and CT-derivedmuscle radio-density in patients with upper GI cancer"* by *M. Ramage (Student)*, Neil Johns, Christopher D.A. Deans, James A. Ross,Thomas Preston, *Richard J.E. Skipworth (Supervisor)*, Carsten Jacobi, and Kenneth C.H. Fearon. This study was conceived by all of the authors. I carried out planning, literature search, data collection and analysis, composition, and editing of the work.

# Acknowledgements

Without the help of a great number of people, this research project would not have been possible.

I wish to thank:

Novartis and ESPEN for providing the funding to allow me to carry out this project with my full concentration.

The late Professor K. C. H Fearon; for inspiration and for leading me through the finer points of cachexia and academic writing.

Mr Richard Skipworth; for endless patience, understanding what I was thinking and how to put it better, and for steering things forward when it seemed without direction.

Professor Steve Wigmore; for stepping in to help after Prof Fearon sadly passed away, and for keeping me moving along.

Dr Carsten Jacobi; for his great help with all forms of biochemical and data analysis, and for ensuring we got the data.

My long-suffering wife Mrs Stacy Ramage; for putting up with evenings in the study and for cracking the whip when things seemed to be slowing.

# Abstract

Cancer cachexia is a constellation of symptoms affecting many cancer patients as their disease progresses. These include loss of muscle mass as well as function, and the utility of computed tomography (CT) in defining this muscle loss has come to the fore in recent years. It is not clear, however, the degree to which CT can be relied on when defining cachexia, interpreting Quality of Life (QoL) metrics, and how this relates to biochemical and functional assessments of skeletal muscle clinically.

Accordingly, the aim of this work was to assess the relationship between CT body composition analysis (CT-BCA) measurements, the consensus cancer cachexia definition, QoL metrics, biochemical, and functional assessments of skeletal muscle.

The hypothesis was that there would be a relationship between CT-BCA measurements and muscular assessment, and that this could be used to inform future research directions.

Patients for this study were recruited from a single tertiary referral centre and were identified through cancer MDTs, and elective admissions. Blood was taken, and CT scans performed, as part of routine clinical work. QoL metrics and functional assessments were performed pre-operatively. Additional blood samples, and Rectus Abdominis muscle samples were obtained intra-operatively. Rectus samples were snap-frozen in optimal cutting temperature compound (OCT) and stored at -80C before being sectioned and fibre diameters measured using an automated process. Muscle protein content was measured using a standard bicinchoninic acid (BCA) method. CT analysis was performed using validated semi-automated software. Statistical analysis was performed in R.

194 patients were recruited into 3 groups (n,M:F): Live donor nephrectomy (LDN) (53, 24:29) (healthy control), Vascular (AAA) (52, 44:8) (non-cancer control), Upper GI cancer (UGIC) (89, 62:27).

Using published criteria utilising both CT-BCA and body mass index (BMI) cut-points, the prevalence of sarcopenia was (n, %): LDN (21, 39.6), AAA (29, 55.8), UGIC (42, 47.2). Cachexia according to published cancer cachexia consensus definition criteria was present in (n, %): AAA (11, 21.2), UGIC (53, 59.6). The

prevalence of patients meeting cancer cachexia definitions in the vascular cohort was unexpected, and may represent a previously unrecognised component of this disease. Using the healthy control group (LDN) to define population-specific cut-points in order to assess prevalence of sarcopenia produced values which excluded the majority of patients.

Differences in QoL metrics were noted between patient groups, with better values noted in LDN patients as could be expected (Mean Overall QoL (lower number indicates better QoL): LDN 6, AAA 30.1, UGIC 29.0). There were, however, few statistically and clinically significant differences in reported QoL metrics or in objective functional measures when patient groups were segregated by CT measures. This may represent the preservation of QoL in a pre-operative cohort; patients sufficiently fit for surgery are likely to have good function and thus QoL, or it may represent a lack of sensitivity of QoL questionnaires to detect aspects or areas of concern to pre-operative patients.

An initial analysis of protein content and CT variables suggested a relationship between skeletal muscle radiodensity (SMD) and protein content. On inclusion of the entire cohort, however, there was no strong demonstrable relationship between CT variables and muscle protein content. This continued to be the case after segregation by CT and BMI cut-points. Attempting to define "normal" muscle protein content utilising the healthy control group was negated by the wide range of measured values across all cohorts: (All,M,F microg/mg wet weight) LDN (44.45-335.4, 59.59-303, 44.45-335.4), AAA (42.4-312.96, 42.4-300.33, 62.63-312.96), UGIC (30.99-314.82, 30.99-314.82, 31.84-250.95).

Muscle fibre cross-sectional area (CSA) was investigated graphically initially. Using this technique, it was possible to partially separate groups of muscle CSA using CT. When using more mathematical methods, however, it was not clear that these separations were significant. Additionally, the derived weighted mean value did not demonstrate a strong relationship with CT variables.

Using anastomotic leak following Ivor-Lewis Oesophagectomy as a surrogate for severe inflammation allowed investigation of this mechanism of cachexia development. Comparison of pre-operative with post-operative scan results produced changes in CT variables which may further confound interpretation (mean skeletal muscle area (SMA) pre- vs post-operative: Male 154.4 vs 162.9, Female 85.4 vs 122.9, mean SMD pre- vs post-operative: Male 32.1 vs 28.1, Female 36.6 vs 27.7). These suggest a possible underlying inflammatory reason for changes seen late in cachexia, and a possible masking of early deterioration in muscle mass.

Currently-used CT cut-points for sarcopenia find higher than expected prevalence in healthy groups, and whilst population-specific values should be derived, they may not always represent a true reflection of muscularity in all groups. Pre-operative patients of all types have globally preserved function and QoL, and more sensitive

metrics are required for this group of patients. CT changes may not reflect QoL reduction until the disease is advanced. The relationship between CT muscle variables and individual components of biochemical muscle composition is unclear and further consideration of alternate muscle constituents such as the nucleus and the sarcoplasmic retuculum is warranted. Inflammation has a marked impact on CT variables, and interpretation of CT variables should include an assessment of systemic inflammation. The current linear paradigm relating muscle mass to function and hence to patient outcome may need to be reconsidered, and this will potentially have an impact on informing the design of and recruitment to future cachexia intervention trials.

CT is a useful tool in the assessment of skeletal muscle and the variables provided can help to indicate prognosis. CT does not, however, reliably inform about QoL; patient function; or muscle constituents; and should not be used in isolation for patient assessment.

# Lay Summary

Cancer is a common disease affecting many patients over the course of their lives. As the disease progresses, there is often loss of muscle - this is termed muscle wasting - and as this progresses the patient may develop a condition called *"cachexia"*. This condition was first recognised by the Ancient Greeks who understood that it represented a state where the patient was likely to have a poor outcome and die relatively rapidly.

More modern definitions of cachexia use CT scanning to measure the amount of muscle present, and use thresholds to say whether patients do or do not have cachexia. Another measurement obtained from CT scans is the density of muscle (how much of the scan x-rays are absorbed by it) and this has also been shown to relate to poor outcomes for patients with low density muscle.

We looked to find how the published thresholds (which were calculated from a North American study) related to Scottish patients, and to see how many of these patients had low muscle mass (sarcopenia), or low muscle mass with weight loss (cachexia). We were interested in whether these scan measures related to Quality of Life measures, to actual physical function measures (such as how fast patients could walk), and to measures of the component parts of muscle (such as protein, and the size of muscle fibres).

There were 194 patients recruited, in cancer, vascular, and healthy live kidney donor groups. We found cachexia in vascular patients, which was unexpected, and we also found more sarcopenia in the healthy control group than we expected.

Looking into Quality of Life, we found that healthy patients had better quality of life than patients with disease, but that CT scan variables did not seem to relate particularly well to quality of life. We also found that our patients had well-preserved physical function, which would be expected from their pre-operative status.

We then looked into the relationship between CT scan measurements and results of laboratory analysis of protein and muscle fibre size. Although there were some interesting changes in muscle fibre area when we looked at the graphs, these changes disappeared when we looked at them mathematically. When we looked at the relationship between CT scan measurements and laboratory protein measures we found there was not a

strong relationship to be found.

We used a group of patients who had undergone cancer operations and who had developed complications, and used existing scans from these patients to investigate the effects of severe complications on CT measurements. This analysis showed that there was a measurable effect of the complication, and that this effect may be important to consider when analysing CT measurements of other patients.

It seems that CT cut-points from North American studies do not translate well to Scottish populations. It also seems that CT measures are not able to predict Quality of Life scores in pre-operative patients, nor are they able to predict walking speed in the same group. Further, the relationship between CT measurements and laboratory test results is not clear. It may be that the relationship between CT measurements and muscle function is not a straight line and instead follows a curve. This is likely to have an impact on future designs of clinical cachexia trials and on which patients should be involved in these.

CT is useful in helping to predict cancer patient outcomes, but it can not be relied upon to predict patient function and CT should not be used alone for this.

# Abbreviations

| Term | Abbreviation |
|---|---|
| Abdominal Aortic Aneurysm | AAA |
| Angiotensin Converting Enzyme | ACE |
| Angiotensin II | Ang-II |
| Angiotensin-Converting-Enzyme Inhibitor | ACE-i |
| bicinchoninic acid | BCA |
| Body Mass Index | BMI |
| C-Reactive Protein | CRP |
| Causal Network Analysis | CNA |
| Computed Tomography | CT |
| Cross-Sectional Area | CSA |
| CT Body Composition Analysis | CT-BCA |
| Deoxyribonucleic Acid | DNA |
| Dual-Energy X-Ray Absorptiometry | DEXA |
| Eastern Clinical Oncology Group | ECOG |
| Edmonton Frail Scale | EFS |
| European Working Group on the Sarcopenia of Older People | EWGSOP |
| Hand-Grip Strength | HGS |
| Hounsfield Units | HU |
| Ivor-Lewis Oesophago-Gastrectomy | ILOG |
| kilogram | kg |

*(continued)*

| Term | Abbreviation |
|---|---|
| Lean Body Mass | LBM |
| Lean Mass | LM |
| Lewis Lung Cancer | LLC |
| Live Donor Nephrectomy | LDN |
| Magnetic Resonance Imaging | MRI |
| Messenger-Ribonucleic Acid | mRNA |
| metre | m |
| Myosin Heavy Chain | MyHC |
| Parathyroid-Hormone | PTH |
| Parathyroid-Hormone Receptor | PTHR |
| Parathyroid-Hormone-related protein | PTHrP |
| Patient-Reported Outcome Measures | PROMs |
| Physical Functioning | PF |
| Quality of Life | QoL |
| Randomised, Controlled Trial | RCT |
| Reported Edmonton Frail Scale | REFS |
| Ribonucleic Acid | RNA |
| Single Nucleotide Polymorphisms | SNPs |
| Skeletal Muscle Area | SMA |
| Skeletal Muscle Density | SMD |
| Skeletal Muscle Index | SMI |
| Subcutaneous Adipose Tissue | SAT |
| Subcutaneous Adipose Tissue Area | SATA |
| Subcutaneous Adipose Tissue Index | SATI |
| Systemic Inflammation | SI |
| Third Lumbar Vertebra | L3 |
| Timed-Up-and-Go Test | TUG |

*(continued)*

| Term | Abbreviation |
|---|---|
| TNF-related Weak Inducer of Apoptosis | TWEAK |
| Tumour Necrosis Factor | TNF |
| Upper GI Cancer | UGIC |
| Visceral Adipose Tissue | VAT |
| Visceral Adipose Tissue Area | VATA |
| Visceral Adipose Tissue Index | VATI |
| Weight Loss | WL |
| Weighted-Mean Cross-Sectional Area | WM-CSA |

# Chapter 1

# Introduction

## 1.1 Cachexia

The term *"cachexia"* originates from Ancient Greece, from the the words *"kakos"*, meaning *bad*, and *"hexis"* meaning *habit or state*. Indeed, Hippocrates described this condition in the context of cardiac cachexia with the description:

"*The flesh is consumed and becomes water; this follows disease of the spleen, of the liver, and leucophlegmasia and dysentery and severe diarrhoea. As a result of the impurities, the abdomen fills with water; the feet and legs swell, the shoulders, clavicles, chest and thighs melt away. If you begin treatment at the beginning, before the accumulation of water becomes excessive, you must administer purgatives which evacuate water or phlegm ... the regimen of food, drink, exercise and walking will be until the patient becomes thin and dry, but only until the flesh becomes as strong as possible. This illness is fatal, above all through progression of ascites. [Regardless of cause] the treatment is similar; but few survive"*[1]

Importantly, the description of widespread oedema and ascites, together with the description that the flesh will *"melt away"*, matches well with the experience in cancer clinical practice. Indeed, some 50-80% of cancer patients develop cachexia at some point in their journey [2,3]. The importance of this is not an academic finding. In fact, the development of cachexia can have significant consequences for the surgeon, the oncologist, and others involved in patient care. Specifically, it is known that patients developing cachexia are less well able to tolerate surgery and chemotherapy, and are more likely to develop complications from these procedures and interventions, potentially precluding their further management [2–4].

### 1.1.1 Cachexia into the modern era

Moving on from ancient times, in the early 20th century Osler described cachexia in a number of conditions including cancer, and noted that emaciation was part of cachexia though it did not define it alone [5]. A more detailed description was provided by Taylor in 1915 [6], who arranged the causes of cachexia under 2 main headings:

"*1. Absorption of decomposition products, toxins, etc. from primary tumour and its metastasis, and 2. interference with the functions of the various organs"*

Taylor's description of the effects of these disturbances, and particularly with regards to the loss of weight related to this, was shown in a 1980 study by Dewys *et al* [2] who noted the effect of weight loss on patient ability to tolerate chemotherapy.

Following this, and as discussed below, there was interest in body composition analysis. This was initially carried out utilising dual-energy x-ray absorptiometry, with a new concept of *"Sarcopenia"* being coined in the 1990s [7], meaning *low muscle mass*. The concept of measuring muscle mass and muscle loss allowed for more precise definitions of what was meant by cachexia, sarcopenia, and the interplay between the terms.
As time progressed, multiple definitions of cachexia were proposed [8–10] , and the problems inherent in having a lack of consensus as to the diagnosis and categorisation of cachexia became increasingly obvious, as described by Fox *et al* [11]. It was against this background, that a group proposed an international consensus definition of cancer cachexia.

### 1.1.2 International Consensus

In the 2011 consensus statement on cancer cachexia, the authors defined the condition as a "multifactorial syndrome characterised by an ongoing loss of skeletal muscle mass (with or without loss of fat mass) that cannot be fully reversed by conventional nutritional support and leads to progressive functional impairment" [12]. This condition affects up to 80% of cancer patients (depending on the type of cancer) [13]. The clinical impact of this condition may result in patients being unable to undergo treatment for their disease (whether surgery or chemotherapy), and in general results in worse outcomes. The diagnosis of cancer cachexia requires the fulfilment of one of three criteria:

- Weight loss (WL) $>5\%$ over past 6 months (in absence of simple starvation)
- BMI (Body Mass Index) $<20$ and any degree of WL $>2\%$
- Appendicular skeletal muscle index consistent with sarcopenia and any degree of WL $>2\%$

The consensus definition acknowledged that at the time of publication there was *"a paucity of reference values"* but suggested a *"generally accepted rule [of] an absolute muscularity below the 5th percentile"*.
These criteria were investigated and validated by Blum et al in 2014 [14]. The validation showed that patients who fulfilled cachexia criteria had a shorter survival time than those who did not. Additionally, the validation confirmed that those meeting cachexia criteria demonstrated increased symptomatology, and exhibited a lower performance phenotype. More recently, however, a Belgian study [15] investigated the discriminatory abilities of Fearon *et al* in comparison to an earlier cachexia definition by Evans *et al* [16], finding that the earlier criteria showed greater discrimination with regards to overall survival when applied to the same population. The Evans *et al* criteria, however, used DXA values to define "low of fat-free mass index" rather than computed tomography (CT).

Of note, the criteria used in Fearon *et al* were defined by converting DXA cut-points to CT using CT body composition analysis (CT-BCA) work done by Mourtzakis *et al* [17]; however these have since been superseded by BMI-stratified criteria developed by Martin *et al* [18], also based upon CT values.

Additional work subsequent to the international consensus definition attempted to further categorise patients within the cachexia diagnosis by building on the foundation of WL and BMI expressed in the statement[19]. This study utilised outcome data from a series of 8160 cancer patients from Europe and North America, and attempted to utilise clinical measures alone to sub-categorise these patients according to subsequent outcome. The authors used a training set to define combinations of WL and BMI which would allow the assignment of a BMI/WL score, and a validation set to confirm their hypothesis. Although able to define severity grades from 0-4 which appeared to match with survival, the datasets used were retrospective, contained a mixture of tumour types, and had a wide range of WL (including a number of patients who gained weight and were thus excluded). The training and validation samples within this study also came predominantly from the palliative and medical oncological settings, and as such may not reflect the experience of those patients undergoing surgical treatment with a view to cure. The BMI/WL score was validated prospectively, and found to be useful for predicting patient outcome [20], however again this validation cohort was composed of palliative cancer patients and the applicability to a surgical cohort is uncertain.

### 1.1.3 Relationship between Consensus Definition and Other Nutritional Assessment Tools

This consensus definition can be shown to interlink with other nutritional approaches to patient assessment. For example the malnutrition universal screening tool (MUST), which is in routine clinical use [21], records height, weight, and WL as part of the calculation used to assign risk group to the patient under study. These measurements complement those outlined in the consensus definition. Additionally, the patient-generated subjective global assessment [22] also uses degree of WL in calculating nutritional risk score. It includes a self-reported measure of patient function, and as such interlinks with the consensus definition's assertion that cachexia leads to progressive functional impairment. These measures were developed prior to the publication of the consensus definition, and it could be said that the definition is in part based on the principles underlying these tools.

Work which has been undertaken subsequent to the publication of the consensus definition has included both BMI and percentage WL as part of the classification system [19], as discussed above.

### 1.1.4 Terminology

As noted above, there have been numerous attempts to define particular terms in relation to muscle loss in the presence or absence of disease. This terminology was summarised by MacDonald [23], in which article the need for clear terms of definition was argued. To this end, and for the avoidance of doubt and confusion, the current report will use terms as defined here:

1. *"Cachexia"* will be defined in accordance with the International Consensus as laid out above.

2. *"Sarcopenia"* will be defined as having low skeletal muscle mass; that is, having a skeletal muscle index below that of a validated cut-point. The term skeletal muscle index (SMI), and the cut-points to be applied will be discussed below and in Chapter 3.

3. *"Skeletal Muscle Area (SMA)"* is the area of skeletal muscle in $cm^2$ at the level of L3 measured across the entire L3 CT scan slice.

4. *"Skeletal Muscle Index (SMI)"* is SMA indexed for height by dividing SMA by the square of the patient's height in metres.

5. *"Skeletal Muscle Radiodensity (SMD)"* is the mean Hounsfield Unit (HU) measurement of the radiodensity of skeletal muscle at the level of L3 measured across the entire L3 CT scan slice

### 1.1.5 Phase of Cachexia

The cachexia syndrome, as described in the 2011 consensus statement, exists as a continuum extending from no cachexia, through pre-cachexia, into cachexia, and ultimately, refractory cachexia and death [12]. In these phases, the latter 3 were described as having clinical relevance. Pre-cachexia included those in whom early changes in metabolism and clinical signs preceded involuntary WL. The cachectic phase is described above, but excludes those who have entered the refractory phase. This refractory phase is described as cachexia which is clinically refractory because of very advanced or rapidly progressing cancer which does not respond to treatment. In cases with features consistent with the cachexia diagnosis, severity stratification as described above can be useful for prognostication and in discussion with patients. However, use of the scale requires strict vigilance from attending physicians. At the early end of the scale, the difference between "no cachexia" and "pre-cachexia" is subtle and can be difficult to distinguish. Early intervention is advocated in the consensus statement (except in refractory cachexia), and to this end, the future identification of early biomarkers is important to aid clinicians in the diagnosis of "pre-cachexia". At the severe end of the scale, the accurate diagnosis of refractory cachexia is reliant on monitoring the patient's overall clinical condition and the specific

response of their tumour to treatment.

### 1.1.6 Staging and Severity Criteria of Cachexia

Although mentioned in the Fearon *et al* International Consensus definition of cachexia, the stage and severity of cachexia were not strictly defined by any particular parameter. Accordingly, in order to better enable prognosis in cachexia, it was necessary to more closely investigate this.

Using a large dataset (8,160 cancer patients), Martin *et al* were able to investigate and categorise WL and BMI levels that most strongly correlated with reduced survival [19]. The authors found that duration of survival reduced with increasing levels of WL, and that survival was also affected by starting BMI. Across all BMI categories, increased WL led to reduced survival, but those with low starting BMI were less tolerant of WL than those with higher BMI. Finding that degree of WL affects survival lends credence to the suggestion that cachexia has degrees of severity, even within the distinct phases of the syndrome. In order to better quantify the severity of cachexia, scoring systems or reproducible cut-off values below which survival time is impacted upon are required. One technique to stage the severity of existing cachexia is the use of body composition analysis to quantify skeletal muscle index.

### 1.1.7 Clinical Utility of Phase and Staging Criteria

Both the phase and the stage of cachexia can and should be used to identify and stratify patients for inclusion in randomised controlled trials (RCTs). The clinical priority at this juncture will be to identify patients with pre-cachexia in order to institute prophylactic interventions, thus avoiding the recruitment of patients with refractory cachexia and a significantly reduced lifespan. Previous trials in cancer cachexia have been plagued by patient attrition rates as high as 50% and in the future, to extract meaningful results, this situation must be avoided [24].

### 1.1.8 Recent Intervention Trials in Cachexia

The last few years have seen the published results of five phase 3 randomised, double blind placebo-controlled trials in patients with stage 3 or 4 non-small cell lung cancer (NSCLC). In the ROMANA 1 and 2 trials, anamorelin, an oral ghrelin receptor agonist (Helsinn) was able to increase LBM over a 12-week treatment period, but not the co-primary endpoint of handgrip strength (HGS) [25]. The results of the ROMANA

3 extension study (a further 12 weeks of treatment for those patients with a preserved Eastern Co-operative Oncology Group (ECOG) performance status (PS) 2 or less) confirmed drug safety/tolerability and maintenance of weight/symptom improvement, but once again did not demonstrate an improvement in HGS compared with placebo [26]. In comparison, in the POWER 1 and 2 studies, treatment with enobosarm, a non-steroidal selective androgen receptor modulator (SARM) (GTx), was associated with an increase in LBM, but not the functional co-primary endpoint of $>10\%$ improvement in stair climb power after 84 days [27]. These studies support the hypothesis that both ghrelin analogues and SARMs may be beneficial for the amelioration of cancer cachexia, but they also highlight the current difficulties in clinical trial design. Future trials must give consideration to: inclusion criteria, by avoiding the recruitment of refractory patients but ensuring consistency in the identification of patients early in the disease continuum (ROMANA 1/2 required patients to be weight-losing or underweight, whereas POWER 1/2 did not); treatment protocols, including drug dosage and the adoption of multimodal interventions (the MENAC trial incorporating exercise, nutrition and anti-inflammatory medication is currently recruiting and aims to establish a standard of care for cachectic patients, ClinicalTrials.gov Identifier NCT02330926); and outcome measures/biomarker of therapeutic response, particularly with regards to functional improvement.

## 1.2 Pathophysiology Underlying Cachexia

The ability of imaging to assess presence, phase, and stage of cachexia is useful. What is not assessed by imaging, however, are the underlying processes which lead from a previously healthy patient to a cachectic patient. There are multiple proposed mechanisms leading from health to cachexia, including alterations in protein metabolism; alterations in adipose tissue metabolism; alterations in glucose metabolism; systemic inflammation (SI); and neurochemical effects of biochemical mediators.

### 1.2.1 Systemic Inflammation

Previous studies have shown that SI is one of the chief drivers of skeletal muscle wasting in cancer cachexia [28], and that SI can be used to predict outcomes in both operable [29] and non-operable [30] cancers. These include colorectal [31,32] and soft-tissue [33] cancers, but similar effects were seen in a systematic review of all cancer types [34]. SI and the hepatic acute phase protein response are major components of the early metabolic response to surgery ('the flow phase') [35], a treatment that many cancer patients undergo. SI is often evidenced by an elevated plasma C-reactive protein (CRP), and an elevated CRP together with a reduction in plasma albumin levels can signify an expected poor prognosis. This relationship was found to be able to predict outcome in a series of advanced lung cancer patients [36] and was termed the Glasgow Prognostic Score (GPS). Subsequent work allowed expansion of this score to preoperative patients undergoing colorectal resection [37,38], and further work has refined the score to its current form as the modified GPS. This ability to predict outcome following tumour resection has been shown to hold true in differing global hemispheres, and using different methods for measuring inflammation [39]. The repeated value of the prediction score underlines the important nature of SI in cancer cachexia.

#### 1.2.1.1 Effect of Systemic Inflammation on CT-BCA

In their study investigating the association between SI and CT-BCA variables, particularly SMI and SMD, Dolan *et al* [40] used a cohort of 650 colorectal cancer patients undergoing resection to show this relationship. Dolan *et al* used previously-published cut-points for both SMI [18] and for SMD [41] having assessed their data and chosen the most suitable values. Dolan *et al* found that there was indeed a significant relationship between CT-BCA and SI as measured by the modified Glasgow Prognostic Score (mGPS), in that those with higher mGPS had lower SMI and SMD. It was not possible to investigate the magnitude of the relationship

using this study design, but this aspect of CT-BCA and SI is one which should be analysed.

In order to ascertain the degree of influence of SI on CT-BCA variables, and with particular regard to the muscle components of CT-BCA, it is important to construct a model with which to perform the investigation. As mentioned above, the use of murine models in cachexia research is fraught with problems. These relate to the relative volume of tumour burden when compared to animal size, to the rapidity of tumour growth, and to tumour behaviour when induced. As such, it would be better to construct a human model of SI in the presence of cancer and cancer cachexia where possible. Understandably, there are ethical concerns around inducing severe SI in humans and particularly in humans with cancer and cancer cachexia.

On this background, using a human model requires that the condition under investigation be one which is known to exhibit a degree of SI such as oesophageal cancer [42]. Additionally, to further add to the inflammatory burden, including patients who had not only undergone operative resection but anastomotic leak would allow assessment of changes in CT-BCA in the presence of severe and sudden SI over and above that induced by the tumour. Anastomotic leak after Ivor-Lewis Oesophago-Gastrectomy (ILOG) has an incidence of around 11% in some series [43]. Additionally, the nature of ILOG means that the surgical anastomosis is constructed within the chest. As such, any inflammatory response arising from breakdown of this anastomosis and impacting on CT-BCA is a reflection of SI rather than local inflammatory changes. Further, CT is often used in the diagnosis and follow-up of such anastomotic leaks [44], meaning that there should be CT scans available from multiple time-points, including at time of diagnosis of disease, at time of diagnosis of anastomotic leak, and at time of follow-up. These factors make patients suffering anastomotic leak after ILOG an ideal population in which to investigate the magnitude of effect of SI on CT-BCA; whether this effect would be visible over a short time-frame; and whether any observed changes follow a pattern which could be explained by current knowledge of SI.

### 1.2.2 Protein Metabolism

Previous studies on muscle protein synthesis and turnover have suggested that the presence of cancer significantly reduces the rate of protein synthesis [45,46], resulting in low muscle mass. The effect of feeding on rates of protein synthesis and breakdown were investigated using labelled amino-acids in a study by van Dijk *et al* [47]. This study showed that in pancreatic cancer patients with cachexia, basal protein turnover was elevated relative to healthy controls. Feeding appeared to improve the overall protein balance in cachectic patients through the mechanism of reduced protein breakdown, whilst protein synthetic rate

remained unchanged. This contrasted with the healthy control group, in whom an equivalent relative increase in post-prandial anabolism took place, but in whom both protein synthetic and breakdown rates were increased in response to feeding. Thus, post-prandial anabolism was similar between cancer patients and controls but differences in protein balance appeared to occur through different mechanisms: reduction in catabolism in cancer patients, and an increase in both anabolism and catabolism in controls. Additionally, Deutz *et al* [48] found that the amino-acid and protein content of food preparations administered and ingested was important when considering muscle synthetic rates. Specifically formulated medical food with controlled protein, leucine, carbohydrate, and fat content increased anabolic rates in cancer patients in contrast to standard food preparations, suggesting a potential role for such supplements as part of a multi-modal cachexia intervention. Measurement of protein synthetic rates in previous studies have potentially been subject to confounding factors inherent in the isotope labelling process. For example, infusions of labelled amino-acids, specifically leucine, may increase protein synthetic rates due to the sudden increase in the presence of these amino-acids if using a flooding dose technique. This is less of a problem using primed constant infusion tracer methods. However, in either technique, patients are frequently immobilised and fasted during the infusion and measurement process. This does not represent normal functioning and thus may not reflect normal protein metabolism. The main drawback to these measurements though is the short-term nature of the observation. To counter this, MacDonald *et al* [49] designed a human protocol for a method of radio-isotope labelling with heavy water to allow an analysis of protein synthetic rates over the course of 13 days, which more closely reflects the natural conditions of muscle metabolism. Following this method, the same group investigated the differences in myofibrillar protein synthesis rates between cancer patients and controls [50]. Perhaps surprisingly, the study performed did not show the expected differences in synthetic rates between the cancer and the control groups. Instead, they found a small but potentially important mismatch in protein synthetic and breakdown rates in the cancer group. In comparison with controls, in whom the rates of synthesis and breakdown were balanced, there was a 2.6% difference between synthesis and breakdown in the cancer patients. This led the group to suggest that although the difference is small, it may be sufficient to account for the previously noted reduction in muscle volume and protein content in pancreatic cancer patients. The results of this study would suggest that the focus for therapeutic intervention in cancer cachexia should be on the reduction of protein breakdown rather than the promotion of protein synthesis.

Another possible mechanism underlying muscle wasting relates to inter-organ nitrogen transfer. It has long been known that catabolic states such as those found post-trauma can result in skeletal muscle protein breakdown to aid transfer of nitrogen to the abdominal organs including the liver [51,52]. This increased

nitrogen availability may in part be explained by an increase in liver protein synthetic rate [53], which includes the production of cytokines and other pro-inflammatory mediators [54]. The alteration of skeletal muscle protein turnover homeostasis, with a move towards reduced synthesis and maintained breakdown rates [55,56], may result in a reduction in skeletal muscle mass which may in part be explained by these mechanisms coupled with a noted increased inflammatory state seen in cancer patients [57,58]. Accordingly, investigations querying the relationship between muscle protein content and SI may yield insights into clinically-relevant biochemical findings.

#### 1.2.2.1 The Assessment of Muscle Protein Content

An assumption underpinning CT-BCA in cachexia quantification is that there is a relationship between CT variables and muscle constituents. There have been studies looking at the physiological basis behind SMD and muscle fat content [59], and work has been done to ascertain the effect of contrast media on CT-BCA variables [60,61], again underpinning the proposed relationship between CT-BCA and muscle components. Whilst there are multiple component parts of skeletal muscle, one part which is possible to test for is that of protein.

The protein content of human muscle in *"reference man"* was derived from experiments measuring total body nitrogen [62]. These experiments were performed in the 1950s, and used 3 samples measuring 0.5-2.0g of skeletal muscle from 4 males killed in *"street accidents"*. These males were aged 21, 27, 40, and 43 years old, but the authors do not describe any anthropometric data or underlying conditions associated with these victims, and do not mention the presence or otherwise of malignancy or other wasting diseases. The conversion from tissue nitrogen (in g/kg) to weight of protein was calculated using a multiplication factor of 6.25 as described by Janney [63]. In the article quoted however, Janney describes how the factor of 6.25 is derived from an assumption that 16% of muscle protein is nitrogen. Janney also describes an increased percentage of muscle protein due to nitrogen in *"higher animals"* of up to 16.7%. Nevertheless, this technique underpins the muscle protein content quoted in *"standard"* reference tables [64].

Accordingly, the protein content of skeletal muscle in *healthy*, *non-deceased* individuals as assessed by modern analytical techniques is unknown. Whilst clearly a *whole-body* nitrogen measurement may more accurately delineate muscle protein content than a copper reduction technique, it is unlikely in the modern era to find *healthy*, *deceased* subjects who have been adequately phenotyped (outwith a capital punishment regime) and the ethics of utilising such samples in the numbers required to reduce the chance of error are questionable.

The assessment and classification of cachexia is important for patient prognostication and treatment guidance. Modern cross-sectional imaging techniques, combined with software planimetry, have been proposed as one

method for the assessment of skeletal muscle and fat volume in cachectic patients. In this technique, the anatomical limits of different tissue areas are defined by radio-density. measured in HU. Lean mass (LM) and total fat mass can then be predicted by calculating skeletal muscle and fat areas at the level of the third lumbar vertebra (L3), and extrapolating these values to the whole body by using validated regression equations [17]. Increased fat infiltration of skeletal muscle (myosteatosis) results in lower SMD on CT [59]. Low SMI [18], as assessed by CT, has been shown to be associated with poor prognosis in a wide range of solid epithelial malignancies. Moreover, reduced SMD is also thought to be an independent predictor of adverse outcome in respiratory and GI cancer patients [65]. However, it is not known whether reduced SMD may also reflect variation in skeletal muscle protein content. The relationship between skeletal muscle protein content and clinical phenotyping has been investigated by Johns *et al* [66]. In this study, the rectus abdominis muscle biopsies of upper GI cancer patients were assessed for protein content against a number of clinical cachexia phenotypes (I - WL >5%; II - WL >10%; III - Low muscularity on CT without WL; IV - Low muscularity AND >2% WL). The investigators found a significant difference in muscle protein content in those in classes I and IV of their cachexia definitions compared to those who were not. The assumed association between WL and muscle protein content serves to underpin a relationship between biochemistry and clinical phenotyping.

#### 1.2.2.2 Skeletal Muscle Fibre Cross-Sectional Area

Human muscle is composed of multiple fibres which can be classified according to their speed of contraction - giving FTIIa (fast) and Non-FTIIa (slow) fibre types. The component parts of the fibres - in particular the Myosin Heavy Chain (MyHC) - have been shown to be universally affected by cancer cachexia irrespective of type of MyHC [67]. Additionally, it was noted in a paper by Johns *et al* [66] that differences in CSA of muscle fibre types could be elicited in cancer patients depending on the clinical classification used to determine *"cachexia"* or *"no cachexia"*. The researchers found that a combination of WL and CT-BCA variables was of most utility in defining the patient phenotype with greatest change in muscle fibre CSA. The importance of this finding is that it illustrates the combined utility of both clinical and imaging-based measures to define cachexia, and shows the need to combine these for a fuller understanding of the underlying changes seen in human muscle.

In addition, changes in SMD have been shown to be of prognostic import in cancer and cancer cachexia [18,68]. There have been investigations into the components of skeletal muscle which contribute to radio-density [59]. This review notes reduction in muscle attenuation with advanced disease, with obesity, with age, and with degenerative conditions. Although Stephens *et al* [69] showed an increase in intramyocellular lipid droplets in

cancer patients, there have been few other studies investigating individual muscle components.

What is also worth considering is the contribution of the cell wall to the radio-density of tissue. Although the contents of individual cells contribute a greater volume per cell than the cell wall, it may be that an increased number of smaller cells, as demonstrated by Johns *et al*, could serve to increase the tissue radio-density by having a proportionally greater mass of cell wall.

Accordingly an investigation into muscle CSA, which will include a measure of cell wall, should help to answer whether there is a strong relationship between SMD and this neglected component of skeletal muscle.

### 1.2.3 Mediators of Cachexia

Moving on from underlying pathophysiological mechanisms leading to cachexia, it is worth considering mediators leading to these processes. Multiple possible mediators of cachexia have been proposed, falling into the general categories of:

- Neuro-endocrine stress response
- Tumour products

Investigation of mediators within these categories using animal models, however, is subject to problems. Each animal model appears to have a different predominant mediator, meaning that generalisability is reduced. Large-scale human studies are lacking, and with incomplete crossover from animal models to humans, this is a hindrance to progress in the field. Additionally, (and as seen in studies of sepsis), the complexity and redundancy of mediator cascades have precluded cause and effect intervention studies.

SI has been shown to be involved in the cachexia process [28], as discussed above. Indeed, there have been multiple studies investigating the effect of elevated concentrations of acute phase response proteins on cachexia and patient survival [70,71], each demonstrating a relationship between SI and worsening cachexia with associated decrease in patient survival. Cytokines involved in SI have been found to be elevated in cachexia [72], and these include interleukin (IL)-1, IL-2, IL-6 and IL-8, as well as tumour necrosis factor (TNF)-alpha and interferon-gamma [72]. Additionally, the Ubiquitin/Proteasome pathway has been investigated as a mediator of cachexia due to its effect on protein degradation within skeletal muscle [54]. These molecules have become accepted as mediators of cachexia, and some newly-described potential mediators are discussed below.

#### 1.2.3.1 Parathyroid-Hormone-related Protein (PTHrP)

The role of Parathyroid-Hormone (PTH) and Parathyroid-Hormone-related protein (PTHrP) in cachexia was described by Kir et al [73] in 2014. In this paper, the team investigated the role of excessive thermogenesis and the browning of adipose tissue in cancer cachexia in a Lewis Lung Cancer (LLC) murine model. Using a combination of cell culture and knockout methods, the group could show that inhibition of PTHrP in tumour-bearing mice markedly reduced the degree of muscle atrophy and fat browning in these mice compared to controls. Interestingly, whilst the injection of PTHrP into previously naïve tumour-bearing mice resulted in significant muscle atrophy, the injection of the protein into healthy mice did not. This led the team to

conclude that whilst PTHrP has a role to play in the excessive thermogenesis and muscle atrophy of cancer cachexia in the LLC model, it must act with a co-factor as yet undetermined. The study also found a reduction in LM in human cancer patients with increased PTHrP. This observation was unrelated to caloric intake or to SI as measured by serum CRP concentrations. These results agree with previous work on human cancer patients [74], and the possibility of a co-factor requirement for cachexia development may explain why patients with primary hyperparathyroidism do not develop muscle wasting. Following on from this initial study, the group then investigated the effect of Parathyroid-Hormone Receptor (PTHR) presence or absence in knockout mice undergoing 5/6 nephrectomy to simulate renal failure-associated cachexia [75]. They demonstrated that removal of PTHR from the adipose cells greatly reduced not only browning of the fat, but also the rate of muscle loss in these mice, despite being unable to fully deplete the receptor from the skeletal muscle. In further studies using the LLC model, the group similarly could demonstrate an apparent protection from the development of cachexia in mice whose adipose tissue was depleted of PTHR. It remains to be seen the degree to which fat-muscle crosstalk affects the phenomenon demonstrated by PTH but with the encouraging murine experience with anti-PTH antibodies [73] this may yet represent a possible therapeutic avenue to be explored.

#### 1.2.3.2 TNF-related Weak Inducer of Apoptosis (TWEAK)

The discovery of TNF-related weak inducer of apoptosis (TWEAK) by Chicheportiche et al [76], and subsequent work by Dogra et al in 2007 of the effects of TWEAK on skeletal muscle [77], has prompted ongoing investigation into the precise role of TWEAK on muscle wasting. Discovery of a TWEAK receptor [78] and up-regulation of TWEAK in both murine and human hepatocellular carcinoma [79] has led to increasing interest in the role of the TWEAK-Fibroblast growth factor-inducible 14 (Fn14) axis in human cancer and cachexia [80]. Studying healthy individuals, Raue et al [81] investigated the induction of both TWEAK and Fn14 in human skeletal muscle following either resistance or running exercise. Taking serial muscle biopsies, the team measured TWEAK protein and mRNA levels and found them to be elevated in the recovery period following exercise. Interestingly, there was a rise in Fn14 mRNA and protein induction suggesting that, whilst the presence of TWEAK within skeletal muscle is involved in metabolism and turnover, the induction of its receptor Fn14 has more of an effect on skeletal muscle metabolism and turnover in the post-exercise period. Using cultured myotubes, Bhatnagar et al [82] investigated the effect of TWEAK on cell atrophy. They found that the addition of TWEAK to the cell cultures resulted in increased expression of factors previously found to be involved in muscle wasting, such as MuRF1, and Beclin 1. To confirm the action of TWEAK, the team used an autophagy inhibitor prior to TWEAK incubation and found reduced levels of autophagy. The group found

activation of both caspases and NF-KB in response to TWEAK incubation, which led to their speculation that TWEAK induces autophagy through a number of possible pathways. An interesting discovery of the effect of anti-Fn14 antibodies on tumour-bearing mice has additionally made the field slightly more complicated [83]. Using murine models to test the effects of anti-Fn14 antibodies on Fn14-bearing tumours, Johnston et al noticed a reduction in the rate and severity of cachexia exhibited by these mice. Using knockout mice for both TWEAK and Fn14, the investigators found that administration of anti-TWEAK antibodies did not have the same reduction in the rate and severity of cachexia development, leading them to conclude that it is the expression of Fn14, rather than the secretion of TWEAK, which is more important in cachexia. These findings in both healthy humans and murine cancer models suggests that there is a close relationship between TWEAK and Fn14, and that Fn14 may be of increased relevance in skeletal muscle metabolism.

#### 1.2.3.3 Angiotensin II (Ang-II)

In their investigation of the effects of Angiotensin II (Ang-II) on insulin-like growth factor, Brinks et al noted a marked loss of weight in rats infused with Ang-II and that this effect was reduced in rats given losartan [84]. In humans, reduced WL in cardiac cachexia was also noted in a heart failure group given enalapril [85]. Following this, the research group including Tisdale et al undertook multiple studies of the effects of angiotensin-converting-enzyme inhibitors (ACE-i), showing both reduced protein synthesis [86] and increased protein catabolism [87] in murine myotubes. In their review of the effects of Ang-II, Yoshida et al [88] discussed the effects of the hormone initially in the context of cachexia associated with congestive heart failure (CHF) and chronic kidney disease (CKD). It was noted within these populations that there were high levels of circulating Ang-II, and that those patients taking angiotensin-converting-enzyme inhibitors (ACE-i) did not lose as much weight as others in the group. Extrapolation from this non-cancer group encouraged investigation in a mouse model, again suggesting that Ang-II has a role in the propagation of SI and the promotion of the cachectic state. Following on from this, work recently done by Penafuerte et al [89] has used an innovative casual network analysis technique (CAN) to identify molecules likely to be acting as mediators or regulators of the cachectic process. This involves the assessment of multiple cytokines in plasma, as well as their upstream regulators, and the mRNA segments related to these. The CAN techniques allow a modelling of complex, multi-faceted systems of interacting mediators to be reduced to more readily understandable schematics based on probabilities of association. This analysis gave Ang-II as a master upstream regulator of cachexia, and specifically pre-cachexia (where patients have not yet deteriorated sufficiently to meet previously-published cut-offs associated with worsened survival [18]). This prediction was validated in the patient cohort by showing

a negative correlation between Ang-II levels and survival. At the genetic level, Johns et al [90] investigated a cohort of cancer patients, looking at single nucleotide polymorphisms (SNPs). Comparing patients with >2% WL and low SMI with weight-stable patients, there were significant correlations between SNPs coding for angiotensin converting enzyme (ACE) and cachexia. Penafuerte et al [89] hypothesised that Ang-II is among the major molecular mechanisms driving cancer cachexia, and it is to be hoped that this may provide another possible therapeutic avenue for a commonly used medication in the form of the ACE-inhibitor. Whilst not specifically a study of cancer cachexia, a retrospective observational study observed that patients taking enalapril for heart failure were at lower risk of WL and demonstrated associated improved survival [91]. These results would seem to support related hypotheses regarding the potential clinical benefit of ACE-i. Although incompletely understood, the role of ACE and Ang-II in the molecular mechanism of cancer cachexia remains relevant and an exciting possible target for intervention.

## 1.3 Imaging in Body Composition Analysis

Within both the international consensus definition of cachexia [12] and the European Working Group on Sarcopenia in Older People (EWGSOP) statement on age-related sarcopenia [92] are defined cut-offs below which the patient is considered to have low muscularity. These cut-off values were determined by population studies using two standard deviations below the mean of young healthy males and females to define those with sarcopenia [7]. These cut-offs are expressed in terms familiar to the users of DEXA. In choosing cross-sectional techniques to use for body composition analysis it is important to recognise not only the clinical advantages inherent in each modality, but also the limitations posed by differing techniques [93], as well as the background behind the use of the most common modality: CT.

### 1.3.1 History of CT in Body Composition Analysis

The original definition of *Sarcopenia* was made by Baumgartner *et al* [7] who defined this condition as having a skeletal muscle appendicular mass lower than 2 standard deviations from the mean of a healthy volunteer cohort. This definition was based on the DEXA findings of some 132 Caucasian males and females with mean ages 46 and 41.5 respectively as described by Gallagher *et al* [94]. It is interesting to note that the populations described in each of these studies vary in number, and the method of reporting age in the paper by Gallagher *et al* is of mean and standard deviation rather than mean and range. Accordingly, although Baumgartner *et al* report the age and ethnicity of their participants to be between 18 and 40 years and non-Hispanic white, the article detailing the cohort (Gallagher *et al*) details a wider age range in fewer subjects. This disparity is perhaps slightly concerning but may be the result of ongoing recruitment and sample selection. In any case, the analysis by Baumgartner *et al* provided the foundation for research into muscle wasting using radiological techniques.

As the importance of SMI became increasingly relevant to the field of cancer cachexia, and with the increasing clinical use of CT in diagnostic and prognostic assessment of cancer patients, Mourtzakis *et al* used 31 lung and colorectal cancer patients who had undergone both DEXA and CT to develop an equation whereby CT muscle area (SMA) values and indices (SMI) and DEXA appendicular muscle mass values could be compared [17]. This allowed routinely-performed clinical CT scans to be re-analysed for prognostication and opened the field to those who did not have access to DEXA as part of standard clinical practice. The equation, and the cut-points it produced, were then validated in a larger cohort of cancer patients [17]. The cut-point derived was one below which the patients appeared to have a shorter life expectancy, and seemed to show there was

an important physiological link between SMI and survival.

As briefly touched on above, the values obtained from CT scan interpretation were those of SMA. These values were derived from a single CT slice at the L3 level and are measured in $cm^2$. As seen in routine clinical practice, patients exhibit differing body habitus phenotypes, and thus there is a need to define a characteristic against which to normalise this SMA measure. In a similar fashion to that used to calculate BMI, the patient's height is measured in metres and then squared. SMA is then divided by the resulting figure to give a measurement in $cm^2/m^2$ - the SMI. This figure can then be used to generate inter-individual analyses, having removed a source of confounding.

Following on from the work by Mourtzakis *et al*, Martin *et al* recognised the importance of obesity in the assessment of SMI [18]. Specifically, in order to carry an increased body mass, there must be an increased muscle mass compared to a non-obese individual of similar height. This is analogous to weight-lifters (obese, carrying more weight) having greater muscle mass than non-weight-lifters (non-obese, carrying less weight). Nevertheless, this increased muscle mass does not necessarily represent a trend towards *"normality"*, rather the obese individual may still have a relative paucity of skeletal muscle and still be *"sarcopenic"* despite having a muscle mass greater than the cut-points previously described. Accordingly, and using a cohort of 1,732 lung and colorectal cancer patients, Martin *et al* produced cut-points which were both sex-specific and BMI specific [18]. In addition, the authors used the other information available through CT body composition analysis to produce further physiologically reasonable cut-points. Specifically, Martin *et al* investigated SMD as imaged by CT.

The radio-opacity or radio-density of items when scanned by CT is calculated according to a specific formula devised by Dr Hounsfield [95], and these values have as their reference points both air and water. When scanning human subjects, particular body tissues will produce distinct values measured in Hounsfield Units (HU). On this scale, the radiodensity of water is zero HU, whilst the radiodensity of air is -1000 HU. In theory, an increase in muscle protein should thus result in a measurable and strong correlation with changes in muscle radio-density (SMD). This theory is investigated and in greater depth in Chapter 7.

Unrelated to muscle cell protein content directly, the findings of Martin *et al* relating low SMD to poor outcome in the lung and colorectal cancer cohort discussed above sparked wider interest. Having noted this effect of SMD on outcome, Rollins *et al* [68] investigated this further in a cohort of palliative pancreatic cancer patients, finding that low SMD was significantly associated with worsened outcome in this group. In order to ensure these results were not confounded by alterations in CT scan contrast phase, the Rollins *et al* also investigated whether there was a significant difference in muscle radio-density depending on scan phase.

Utilising routine clinical investigations, they were able to compare multiple CT scan phases and found that although statistically significant differences between the phases under investigation existed, it was possible to use specific mathematical equations to convert radiodensity from one phase to another [60]. This work was highly useful in demonstrating the comparability of multiple CT scan phases, allowing diverse patient groups to be investigated and stratified equally.

#### 1.3.1.1 Importance of BMI Characteristics in CT Body Composition Analysis (CT-BCA)

Within the UK, and across the world, the national and international trend is one of an increasing prevalence of obesity [96]. Given this demographic, which has changed even in the short time since Baumgartner *et al* produced their original findings, it is reasonable to split populations not only by sex, but also to include a measure of whether these individuals are overweight. The paper by Martin *et al* [18] investigated this trend, and produced sex- and BMI-specific cut-points for prognostication in their cohort. They found that in males, there was a difference between low BMI ($<25$) and high BMI males in terms of their SMI cut-points; but not in females, for whom a single cut-point alone gave prognostic value. These cut-points reflected the observed higher SMI in males, and also the observed higher SMI in obese males. It is currently unclear why females should not display the same weight-related differences.

#### 1.3.1.2 Importance of SMI in CT-CBA

As mentioned above the original BCA variable was appendicular muscle mass, which was indexed to height, and was also measured using CT to give CT-BCA. There have been multiple studies investigating the relationship of SMI to outcome in cancers of all types. Carrara *et al* [97] recently found low SMI relating to tumour stage in pancreatic cancer, while Villasenor *et al* [98] encountered a similar finding in breast cancer. This study used a longitudinal analysis over 10 years and concluded that those who demonstrated sarcopenia on preoperative DEXA retained a poorer prognosis even to the end of the follow-up period. Although utilising a less validated technique (psoas area) at a less validated level (L4 vs L3), nevertheless a relationship between low muscle mass and operative complication rate was found. Also using psoas area, Sabel *et al* [99] found a similar relationship in Stage III melanoma patients, with SMI being an independent predictor of worse survival. Indeed, this impact of low SMI has been seen in older patients with cancer [100]; in hepatocellular cancer [101]; in metastatic colorectal cancer [102]; and in B-cell lymphoma [103]. Low SMI has also been implicated in increased operative complication rates in squamous oesophageal cancer [104], and in an increased cardiovascular risk profile in

Korean male surviving cancer patients [105]. These studies, although utilising a variety of methods to assess muscularity, do all point to a significant impact of muscularity on survival.

#### 1.3.1.3 Importance of SMD and Contrast Phase in CT-BCA

An alternative way to assess muscle nutritional response to pharmacological intervention using CT would be to assess muscle *"quality"* rather than *"quantity"*, by measuring the radio-density of skeletal muscle in Hounsfield Units. The effect of low SMD on outcome has been noted in several diseases. In pancreatitis, Grinsven *et al* found a decrease in muscle radio-density during the course of the disease resulted in poorer survival [106]. Similar findings were noted in pancreatic cancer by van Dijk *et al* [107] where in a prospective study involving 199 patients with head of pancreas cancer, medium or low muscle radio-density was associated with worsened survival. Poorer survival was also noted in patients with endometrial cancer studied by Paula *et al* [108], and in palliative pancreatic cancer studied by Rollins et al [68]. In these cohorts, the combination of low muscle index with low SMD appeared to worsen outcome. In the latter study, previously published muscle index and density cut-points [18] were used to stratify patients into sarcopenic/normal and myosteatotic/normal. Both sarcopenia and myosteatosis carried poorer prognosis, but in combination these factors were synergistic in their survival reduction. However, there is some preliminary evidence that SMD may correlate more closely with physical function (PF) in elderly cancer patients compared with SMI [109]. The inference, in line with the observations of Martin *et al* [18], is that low SMD is the result of altered muscle composition at a cellular level, and that protein is replaced by intramuscular fat. However, once again, this assumption may not represent the entire picture. This is discussed in greater depth in Chapter 7.

Similar to SMI, it seems that radio-density cut-points also require specific development for each population under study. Studies performed by differing centres have found differing radio-density cut-points apply to their cohorts [107,110], and a recent review noted similar findings [111] throughout the literature. Increasing interest in muscle radio-density has led to concerns regarding the effect of intravenous CT contrast media on body composition variables derived from scans performed in different phases. Earlier authors did not disclose which CT scan phases were used in their manuscripts [17,18]. However, work carried by van Vuht et al [61] investigated 50 liver transplant patients undergoing triple-phase CT scans and found statistically and clinically significant differences in SMD between non-contrast and contrast-enhanced scan phases, but not in muscle area. Applying the Martin *et al* [18] criteria to these values, resulted in 80% having low SMD in unenhanced scan phases whilst 50% and 38% had low SMD in arterial and portal venous phases respectively. The findings from Rollins *et al* [60] in a population of pancreatic cancer patients was that there was a statistically significant

difference between contrast phases on CT, however there was a linear relationship between these phases which could be translated via simple equations to provide comparable values. In more closely defining the relationship between contrast phase, these authors have moved the literature forward to allow abdominal CT scans performed for any clinical indication to be compared. However, such phase-dependent values will obviously impact on any statistical relationship with PF outcome measures. This problem can be compounded further by other CT technique-dependent variables such as tube potential [112]. Authors are now turning to complex non-linear trimodal regression analysis techniques of entire radiodensitometric muscle distributions to compare with standard CT metrics and lower limb muscle function [113].

#### 1.3.1.4 Controversies in CT-derived Sarcopenia

Concern has been raised, however, in the use of CT scanning for this purpose with specific regard to the EWGSOP definition of sarcopenia. This definition includes a requirement for the quantification of muscle function as well as volume. Safer and Safer have repeatedly questioned the use of CT planimetry alone to assess sarcopenia, stating the requirement in the EWGSOP statement to include muscle function assessment[114]. Other issues raised by Safer and Safer, and Cintosun *et al*, include the specific lumbar levels used for analysis, comparison with other imaging modalities, and differences between scanners as possible confounders [115,116]. In response to these questions, the authors of the articles criticised acknowledge the absence of muscle function assessment but draw attention to pragmatic reasons for this: the pathophysiological differences between sarcopenia and cachexia; the large studies validating CT as an imaging modality; and the standardisation of modern CT scanners [117–120]. In addition, the specific impact of low muscle volume on prognosis and morbidity [18], as well as the recent review confirming this phenomenon across the reported literature [111], support the use of CT-derived cut-offs as markers for increased morbidity and mortality in cancer patients. However, equally, it should be remembered that low muscle volume is a binary measure that does not acknowledge the dynamic process of skeletal muscle wasting. Furthermore, CT-derived sarcopenia may not equate with muscle protein content (as discussed in Chapter 7), therefore raising questions as to its utility as an outcome measure in interventional trials. In a recent editorial and linked paper, Baracos [121] and Rutten *et al* [122] argue that a recent trend for utilising psoas major alone for assessment of skeletal muscle in the context of sarcopenia has significant limitations. Specifically, the relationship of psoas area to whole body muscle volume was unclear, as was the relationship of psoas area to overall survival. Additionally, the cut-points derived by Martin *et al* [18] were from a Canadian cohort which from the Alberta census consists of a 70% European origin population [123]. It is known that for DEXA scanning, cut-points for defining sarcopenia are different

in European populations and Asian populations. Specifically, the EWGSOP [124] defines low muscle index in European females below 5.5 kg/m$^2$, whereas in a Korean population Kwon *et al* [125] found the cut-point to be 4.4 kg/m$^2$. Similarly for CT derived variables, Fujiwara *et al* [126] found different cut-points in a Japanese cohort to those developed in the Canadian group. It therefore seems that population-specific cut-points for SMI need to be derived in any trial population before assessing muscle function. Some studies have shown that it is sarcopenic obesity that carries the worst clinical risk [127], whereas more recently, the negative prognostic impact of subcutaneous fat [128,129] has been demonstrated.

#### 1.3.1.5 Adipose Tissue in CT Imaging

Other variables obtained from CT analysis include the area and radio-density of visceral and subcutaneous adipose tissue (VAT and SAT). These have garnered attention as potential predictors of survival in cancer patients [128]. In their study, Ebadi *et al* investigated the relationship between visceral and subcutaneous adiposity and the effect of these on survival in sarcopenic and non-sarcopenic cancer patients; reporting a significant relationship between low subcutaneous adiposity and worse survival after adjustment for known survival predictors. Although interesting, the majority of these patients had stage IV disease, and the utility of VAT and SAT values and cut-points for clinical decision-making requires further study.

### 1.3.2 Magnetic Resonance Imaging (MRI)

Magnetic resonance imaging (MRI) is an increasingly attractive form of cross-sectional imaging, and is now considered equivalent to CT as the gold standard imaging methodology for skeletal muscle volume analysis [12,92,130]. MRI scanning has been used in sports medicine [131] and in muscular dystrophy [132] to monitor the effect of injury or disease progression on the volume of scanned muscle. Recent advances in automation have shown this technique to be feasible in a whole-body context, allowing the definition and direct measurement of total skeletal muscle volume within a patient [133]. In contrast to CT scanning, which uses a single slice and algorithmic conversion to a height-based index [18], direct measurement using MRI has clear advantages. Firstly, the entire volume of scanned muscle is measured directly rather than inferred or calculated. Secondly, there is no need for ionising radiation. Thirdly, it should be possible to use MRI to determine water and fat levels within a particular muscle, as myosteatosis has been shown to have an adverse prognostic effect on patients with pancreatic, gastric, and lung cancer, as well as non-cancer inflammatory conditions [68,69,134–136]. Disadvantages to MRI scanning do exist, however, and include the labour-intensive nature of interpreting each

scan; the cost in terms of the scanner and the staff required to run the scans; and the demand on patients – each scan takes 20-60 mins in a claustrophobic scanner environment with strict requirement for exact repositioning for reproducibility. Additionally, CT scanning is routinely used in clinical cancer practice in contrast to MRI, and so additional scaning is not required for CT-BCA analysis unlike MRI.

Overall, the field of contemporary body composition analysis is highlighting the the importance of low SMI as an independent adverse risk factor in cancer patient survival. As sarcopenia definitions continue to require measures of muscle function, it may be time to separate the syndrome of sarcopenia from isolated low muscle volume, and rename the latter condition myopenia [137].

### 1.3.3 Assessment of Physical Function (PF) Outcome Measures

The identity of the best PF outcome measure to relate nutritional outcome measures, including CT, is unknown. There is a lack of PF tests that specifically analyse upper abdominal/L3 muscle activity. Equally, it is an unproven assumption that L3-CT should, in some way, correlate with either targeted assessments of isolated limb strength and power, or complex whole-body assessments of PF (e.g. Timed-Up and Go-Test (TUG)) [138]. This is further investigated in Chapter 6. Some have previously advocated physical activity meters as devices to measure global patient function in the free-living environment [139,140]. The advantage of such devices is that they offer multiple outcome measures from a single application. However, once again, for the purposes of clinical trial design, a single outcome measure is required to be pre-chosen as co-primary endpoint. HGS has been used widely in nutritional studies in this role, and has been validated in various populations [141,142]. However, as described previously, it was unsuccessful in the ROMANA studies. From a clinical perspective, it might seem that measures of upper limb PF might be less meaningful or less affected by pharmacological intervention when compared with measures of lower limb PF, which in turn might dictate activities of daily living and overall exercise limitation. However, stair climb power was unchanged in the POWER studies. Despite these negative findings, and with no clear relationship between SMI or SMD and PF, studies continue to report CT-BCA findings relating to poor patient outcome, as mentioned above. Thus, there is a need to understand which PF test relates best to CT-BCA; which PF test relates best to outcome; and whether CT-BCA with or without PF testing can be shown to relate to muscle biochemistry as a means of explaining the outcomes observed.

Another aspect of PF measures, and as mentioned above in relation to CT-BCA, is the normalisation of such measures. As described above, CT-BCA SMI is calculated by taking the skeletal muscle area of the L3 CT

slice and indexing it for height by dividing the area measured by patient height in metres, squared. When considering PF, however, the measures reported above are commonly presented either as raw values (in the case of the timed up-and-go (TUG) test) or having been assessed alongside other measures and combined into a single score which is sub-divided to give a series of categories (such as the Edmonton Frail Scale - dividing patients into Not Frail, Pre-Frail, and Frail)[143].

## 1.4 CT and Patient Status

As described above, cachexia has been defined by international consensus as "a multifactorial syndrome characterised by an ongoing loss of skeletal muscle mass (with or without loss of fat mass) that cannot be fully reversed by conventional nutritional support and leads to progressive functional impairment" [12]. The clinical importance of the latter part of the definition is in the stress on *"progressive functional impairment"*. This can be measured objectively, by techniques such as those proposed by Cruz-Jentoft *et al* [92] in their EWGSOP definition paper recommending HGS and gait speed analysis as measures of muscle function.
Additionally, patient assessment can include measures of QoL including patient-reported outcome measure (PROM) tools to quantify aspects of health which may be affected by disease. These can include measures of symptomatology, and of patient-reported functioning distinct from objective measurements.

### 1.4.1 What Is Function And Why Is It Important?

Patient PF can be described in terms of specific muscle function, such as that assessed by HGS or gait speed, or in terms of a more global function, such as that assessed by Eastern Clinical Oncology Group (ECOG) score [2]. Specific muscle function tests like HGS have the advantage of objectivity in their assessment, allowing for cut-points to be produced and definitions to be written [92]. How well these tests translate into real-world settings is unclear, however. Other tests investigating particular muscle groups, though more globally, include the timed up-and go (TUG) test. This involves the patient rising from a seated position, walking 3m, turning, and returning to their original seated position. It has shown good inter- and intra-observer reliability [144]. This type of test is more pragmatic and easier to perform in a clinic setting with minimal equipment than a HGS test. Additionally, if measured separately, the walking components can be used to measure gait speed [145] which is one of the suggested definitions of sarcopenia in the EWGSOP statement. When investigating the function of patients in a more global fashion, the ECOG score mentioned above allows the clinician to subset their patients into groups depending on function as a whole, rather than as a measurement of a specific muscle set [4]. In this paper, Oken *et al* suggest a scale of ability from 0 (fully active, able to carry out pre-disease activity without restriction) to 5 (dead) allowing not only a comparison of patients between study centres, but also allowing the clinician to monitor patient progress throughout their disease course (see Table 1.1). More recent work by Blagden *et al* has suggested that ECOG scores assessed by patients agree with those assessed by oncologists in the majority of cases [146], suggesting that both doctor-reported and patient-reported functional assessments may have validity. In their study, Blagden *et al* also showed that in line with other previous studies, ECOG

score did correlate with survival in a lung cancer population, whether patient-scored or oncologist-scored. This being the case, the inclusion of patient-reported functional assessment in current and future studies has merit. Additionally, work by Laird *et al* [147] has shown concordance between ECOG score and patient-reported measures of PF as quantified in patient-reported questionnaires. These are discussed below. The inclusion of both patient-reported function and patient-reported symptomatology should provide an increased ability for the researcher to understand and interpret patient PF.

With the increasing age of the population, a concept coming increasingly to the fore is that of frailty [148]. In this position paper, Bergman *et al* discuss the concept of frailty as a specific syndrome and draw from existing literature possible defining features of what could make a patient frail. They note that it is likely to be impossible to clearly separate frailty from ageing, and also note that one component of frailty is that of progressive muscle weakness. This is not dissimilar to concepts encountered in considering the effect of age on sarcopenia, and it is reasonable to suggest that these factors interplay to cumulatively affect individual patients. In their paper discussing the assessment of frailty in acute care, Hilmer *et al* [149] note that controversy exists regarding the definitions of frailty. They argue that despite this, it is important to find some common features which can be elicited by either clinicians or non-clinicians and which can be used to guide interventions and produce a prognosis in the frail. To this end, the group investigated the Edmonton Frail Scale (EFS), which had previously been developed and validated against a specialist assessment of frailty [143]. Hilmer *et al* produced a reported scale (REFS) based on the EFS, though not requiring a TUG to be performed. This was validated against a specialist assessment of frailty in a similar fashion to the validation of the EFS, and allows patients to be classified into *Not Frail*, *Apparently Vulnerable*, *Mild Frailty*, *Moderate Frailty*, and *Severe Frailty*.

Table 1.1: ECOG Score and Definitions

| ECOG Score | Definition |
|---|---|
| 0 | Fully active, able to carry on all pre-disease performance without restriction |
| 1 | Restricted in physically strenuous activity but ambulatory and able to carry out work of a light or sedentary nature, e.g., light house work, office work |
| 2 | Ambulatory and capable of all selfcare but unable to carry out any work activities; up and about more than 50% of waking hours |
| 3 | Capable of only limited selfcare; confined to bed or chair more than 50% of waking hours |
| 4 | Completely disabled; cannot carry on any selfcare; totally confined to bed or chair |
| 5 | Dead |

## 1.5 What Is Quality of Life (QoL) And How Is It Measured?

In his narrative review of QoL [150], Post explores the origins of the concept of *"Quality of Life"* and how the concept has become more central to healthcare since 1948. In it, Post quotes an editorial by Elkinton [151] from which the following is taken:

*"What every physician wants for every one of his patients old or young, is not just the absence of death but life with a vibrant quality that we associate with a vigorous youth. This is nothing less than a humanistic biology that is concerned, not with material mechanisms alone, but with the wholeness of human life, with the spiritual quality of life that is unique to man. Just what constitutes this quality of life for a particular patient and the therapeutic pathway to it often is extremely difficult to judge and must lie with the consciousness of the physician."*

This early idea of the meaning of *"Quality of life"* includes the suggestion that it is unique and individual to each patient, and thus will have to be investigated and quantified on an individual basis to have true meaning. This is clearly very different to such performance metrics as the ECOG score mentioned above, and will require a different technique to elicit.

### 1.5.1 Patient-Reported Outcome Measures

The technique used in evaluating QoL in patients is that of PROMs. As a method of driving clinical trials, this has gained traction recently, and has resulted in the production of an addendum to the original CONSORT statement [152] which includes guidelines on the inclusion of PROMs in trial design and reporting [153]. In it, the authors recognise the increasing use of PROMs to "inform patient-centred care" and detail the points at which the PROMs chosen should be justified and reported. The idea of using PROMs to inform patient-centred care and of involving patients in the trials carried out to investigate their diseases signals a move away from paternalistic approaches towards increased patient autonomy.

### 1.5.2 The Relationship Between QoL and CT

The relationship between between CT body composition variables and QoL has been investigated by Bye *et al* [154] in a lung cancer population. They found non-linear relationships between some aspects of the QLQ-C30 and SMI, and that the SMI had to drop below specific *"break-points"* before a visible relationship was observed. This suggests that there is not a true linear relationship between muscle index and PROM PF, or between muscle SMD and PROM PF, in patients with advanced lung cancer. It is not clear from that particular study, however, what the relationship between CT variables and QoL may be in a preoperative surgical upper gastrointestinal cancer population, or in other populations.

To this end, the current study included the use of validated QoL questionnaires. The EORTC QLQ-C30 with add-on module OG25 questionnaire [155] was chosen due to the main instigative focus of the current study being on patients with upper GI cancer. The base QLQ-C30 contains questions regarding QoL which are applicable to all populations under study, and to demonstrate differences between populations all were asked to complete the OG25 module in addition. The second questionnaire used was the EuorQoL EQ5D-3L questionnaire [156] which was chosen as a general and global QoL assessment tool. Both of these questionnaires have been validated in European populations, and provide insight into global QoL as well as into specific symptomatic and functional performance domains.

# Chapter 2

# Aims

The overall aim of this study was to assess CT-BCA in order to ascertain whether CT-BCA is accurate in assessing a number of assumptions, and its potential validity for future use as an outcome measure in interventional trials.

The study aimed to assess this in a staged fashion:

1. Assessing CT-BCA cut-points
    - Applying published cut-points to recruited patients
    - Deriving a new cut-point from recruited patients
    - Comparing new and published cut-points
2. Assessing patient quality of life
    - Comparing quality of life between patient groups
    - Assessing the utility of CT-BCA cut-points to show differences in quality of life within patient groups
3. Assessing objective patient function
    - Measuring patient function

- Using linear regression to demonstrate a relationship between CT-BCA and function

- Assessing utility of CT-BCA in showing differences in function by CT-BCA cut-point

4. Assessing muscle protein content
    - Comparing skeletal muscle protein content between patient groups using modern techniques

    - Defining a relationship between CT-BCA and muscle protein content

    - Utilising CT-BCA cut-points to show differences in protein content between groups

5. Assessing skeletal muscle fibre cross-sectional area
    - Assessing the difference in cross-sectional area between patient groups

    - Assessing the difference in cross-sectional area between CT-BCA cut-point groups

    - Assessing the relationship between CT-BCA and cross-sectional area

6. Assessing the relationship between systemic inflammation and CT-BCA
    - Defining a human model of severe systemic inflammation

    - Assessing differences in CT-BCA before and after onset of severe inflammation

    - Assessing differences in CT-BCA during and following severe inflammation

# Chapter 3

# Methods

## 3.1 Recruitment of Patients

Patients were recruited to the study from 3 groups:

- An upper GI cancer (UGIC) group included patients with oesophageal, gastric, and pancreatic cancer. These patients were chosen due to their high incidence of cachexia, and because their preoperative staging investigations included abdominal CT scans. Resectional surgery included Ivor-Lewis Oesophago-Gastrectomy (ILOG), thoraco-abdominal oesophago-gastrectomy, thoracoscopic oesophago-gastrectomy, laparoscopic-assisted oesophago-gastrectomy, gastrectomy, and pancreatico-duodenectomy.
- A vascular aortic (AAA) group included patients who were undergoing aortic surgery for aneurysmal disease, including both abdominal and thoracic. These patients were chosen for their expected age and co-morbidity match with the UGIC group, and because their operative planning investigations included abdominal CT scans. Operative surgery included tube, bifurcated, and multi-limb synthetic graft repair. Both thoracic and abdominal aneurysm repair required exposure and incision of the rectus abdominis muscle.
- A healthy live donor nephrectomy (LDN) group who had completed the living donor workup and were donating one of their kidneys. These patients were chosen as a selected healthy group with minimal disease, and because their workup included abdominal CT scans. Nephrectomy was carried out either through a single-incision laparoscopic surgical port or utilising a hand-assisted laparoscopic technique.

Patients in each group were identified independently by the clinical team overseeing their care.

Those in the UGIC group were identified at a weekly multidisciplinary team meeting (MDT) which reviewed all patients referred with upper GI cancer and stratified them into those for resectional surgery and those not. Once identified by the MDT, these patients were approached in clinic and recruited into the study in line with the study protocol as approved by the local research ethics committee (REC).

Those in the AAA group were identified by the vascular team at the time of their admission the day before their aortic surgery, and were approached and recruited as above.

Patients in the LDN group were identified by the transplant surgical team in advance of attendance at their preoperative clinic, and approached at this clinic.

In line with guidance from the REC, all patients were provided with written information, and were allowed to decline recruitment, or to withdraw from the study at any time.

To assess the effects of inflammation on CT-BCA, a database of patients undergoing ILOG between 2012 and 2016 was queried to find those who had suffered anastomotic breakdown. These patients had their CT scans analysed as described below, and phenotyping data was garnered from their medical records.

## 3.2 Sample Size Calculation

This was an exploratory study investigating new relationships between CT-BCA and patient phenotype, and thus a power calculation is difficult to perform *a priori*. In order to generate a potential sample size for ethics validation the outcome of a previous study investigating dystrophin glycoprotein complex dysfunction [157] were used. This study had shown statistically differences in dystrophin glycoprotein complex dysfunction between cancer patients and healthy controls using a sample size of 27 and 14 respectively.

It was felt that aiming for an increase in sample size from 27 to 40 in each group would allow valid comparisons to be made between each group, and accordingly a total sample size of 120 patients was set as a target in agreement with the regional ethics committee.

## 3.3 Patient Phenotyping

Physical parameters including height, weight, and weight loss amount and duration were collected to allow calculation of body mass index (BMI) and percent weight loss from normal. Patients were asked to complete timed-up-and-go test [144], which was timed in segments to allow gait speed calculation [145]. In this test, patients were asked to stand, walk to the end of a pre-measured 3m distance, turn, return to their seat and sit down. Both walking laps were measured, and the gait speed calculated from these.

Patient past medical history was collected, as were lists of medications. From this, the Charlson Co-morbidity Index (CCI) [158] and the Scottish Co-morbidity Scoring System (SCSS) [159]. Although in more common use, CCI gives more weight to conditions now considered controllable (such as the human immunodeficiency virus and the acquired imunne deficiency syndrome), whilst SCSS has been validated in a Scottish population [160] and uses international criteria (such as the New York Heart Association Heart Failure Classification) to stratify co-morbidities. Patients also completed the patient component of the patient-generated subjective global assessment (PG-SGA) [22], and quality of life questionnaires. From the PG-SGA, a patient-reported ECOG score was derived. Patients also completed the Edmonton Frail Scale (EFS) [143], and the reported EFS (rEFS) [149]. At the time of surgery, American Society of Anaesthetists (ASA) score [161] was collected.

## 3.4 Quality of Life (QoL) analysis

During their clinic visit, patients completed the EORTC-QLQ-C30+OG25 questionnaire [155] and the EQ5D-3L questionnaire [156].

### 3.4.1 QLQ-C30

The QLQ-C30 from the EORTC group is a well-recognised, validated questionnaire covering QoL aspects related to cancer patients. The base questionnaire is designed to be generic and captures many domains common to all cancer patients. Additionally, the generic QoL aspects were felt to be useful for comparison with the AAA and LDN groups to demonstrate expected differences according to disease state. The QLQ-C30 also has a modular component which allows tailoring to particular tumour types. Both the At the time of study design the EORTC cachexia add-on for the QLQ-C30 was unavailable, and at present (2019) the questionnaire is still undergoing validation. Accordingly, the OG25 additional component was chosen to cover both the expected oesophageal and the gastric cancer patients. The inclusion of a specific vascular patient-reported outcome measure (PROM) was not performed, as at the time of study design there was only one PROM of that sort available [162] (the SF36 noted in Duncan *et al* [162] is a generic QoL tool) and the further addition of questionnaires was felt to be a burden on the patients recruited.

The QLQ-C30+OG25 was analysed in keeping with the instructions [155], and scores were converted to percentages according to weighting also in keeping with the reporting manual. This conversion was performed using QoLR [163], a quality of life package for R statistical software. A difference in reported QoL of $>10$ points was felt to be clinically significant, as suggested by the QLQ-C30+OG25 instruction manual [155].

### 3.4.2 EQ5D-3L

The EQ5D-3L from the Euroqol group was also administered to patients in clinic prior to surgery. This is a generic QoL PROM and allows patients to score 5 domains in 3 levels from best to worst. The final page includes a 100-point scale for indicating overall QoL at time of administration. Analysis was conducted between the overall QoL scales in each questionnaire as a continuous scale.

### 3.4.3 Timing of Questionnaire Administration

With the data available, it was not possible to accurately ascertain what proportion of patients recruited undertook a QoL assessment before, and what proportion after, any chemotherapeutic regime was undertaken. Upon investigating further, however, it was possile to see a separation of dates between recruitment and operation at around the 50 days mark. Taking this as a possible threshold, differences in symptomatology were investigated to ascertain differences between scores over/under 50-days.

## 3.5 CT Body Composition Analysis (CT-BCA)

Preoperative staging CT scans were analysed at L3 level using semi-automated Slice-O-Matic software v4.2 (Tomovision Montreal-Canada), which defines whole-slice area and radio-density measurements of skeletal muscle, subcutaneous fat, and visceral fat. This technique requires the investigator to select the L3 CT scan slice. Following this, the software utilises HU values to tag areas of the scan image as muscle or other tissues. The total area of the tagges muscle is the SMA (in $cm^2$). This value is the divided by the square of the patient's height in metres to give SMI. Mean HU measurement is taken across the whole area tagged as skeletal muscle across the entirety of the chosen slice. In practice, and as described by Heymsfield *et al* [164], tissues between -29 and 150 HU were tagged as skeletal muscle by the software. Following this a manual review of each slice was undertaken to ensure that extraneous tissue was not erroneously tagged (this review gives the "semi-automated" nature of the analysis). It was then this area of tagged tissue which was used for the measurements detailed above.

At the outset of the research project, training was undertaken in use of the software to ensure appropriate CT scan slice level was chosen for analysis, that the software was being utilised correctly, and that measurements obtained from the software were appropriate. This was performed over 20 CT scans, which were selected by the trainer and then analysed by the trainee.

Skeletal muscle are measurement was indexed for height by dividing skeletal muscle area by the square of the patient height in metres, producing Skeletal Muscle Index (SMI, $cm^2m^2$) [17] and then stratified into *"Normal"* and *"Low"* according to published cut-points [18] as seen in Table 3.1.

It was recognised that multiple software packages are available for CT-BCA, including Slice-O-Matic, ImageJ, and OsiriX. Both Slice-O-Matic and ImageJ have previously been investigated to ascertain whether there is agreement between packages in terms of values obtained from CT-BCA, and have been found independently

Table 3.1: Martin et al Cut-Points

| Patient Type | SMI Cut-Point (cm2/m2) | SMD Cut-Point (HU) |
|---|---|---|
| Low BMI Male | <43 | <41 |
| High BMI Male | <53 | <33 |
| Low BMI Female | <41 | <41 |
| High BMI Female | <41 | <33 |

Table 3.2: CT Phase Conversion Equations

| Phase Conversion | Equation |
|---|---|
| Non-contrast to Arterial | SMDa = 0.973 x SMDnc + 3.747 |
| Non-contrast to Portal Venous | SMDpv = 0.957 x SMDnc + 6.723 |
| Arterial to Portal Venous | SMDpv = 0.979 x SMDa + 3.213 |

to agree[165,166]. Slice-O-Matic has also been compared to OsiriX, and whilst the values obtained from these packages were statistically significantly different, the authors felt that these differences were unlikely to have clinical relevance [167]. The authors of this study did, however, recommend that a single software package was used for CT-BCA analysis, to prevent confusion. Accordingly, as Slice-O-Matic and ImageJ have previously repeatedly and independently been shown to concur in CT-BCA measurements a repeat comparison was not performed, and the Slice-O-Matic software package was chosen for use due to availability, software ergonomics, and user experience.

Muscle radio-density was acknowledged to be an important factor in CT-BCA [68], and CT scan phase was also noted to be of relevance [61]. Accordingly, published equations were used to convert values obtained from arterial and non-contrast phase scans to portal venous phase equivalents [60] as shown in Table 3.2:

## 3.6 Cachexia Definition

As discussed in Chapter 1, the International Consensus definition of cachexia includes 3 criteria for diagnosis [12]. As also mentioned in Chapter 1, and in the Consensus definition, muscularity criteria should be sex-specific and should take in to account the presence of overweight or obesity. The criteria by which patients can be diagnosed with cachexia are presented in Table 3.3, and the CT-BCA cut-points in use in this study are presented in Table 3.1.

Table 3.3: International Cachexia Consensus Definition

| Criteria | Definition |
|---|---|
| 1 | Weight loss >5% over past 6 months (in absence of simple starvation) |
| 2 | BMI <20 and any degree of weight loss >2% |
| 3 | Appendicular skeletal muscle index consistent with sarcopenia and any degree of weight loss >2% |

## 3.7 Weight loss and BMI

As discussed in Chapter 1, the analysis by Martin *et al* [19] relating BMI and weight loss percentage to outcome in a series of cancer patients gives a BMI/weight-loss score which can be calculated for each patient according to where they lie on the chart seen in Figure 3.1. This score was utilised to subdivide patients by BMI/weight-loss category for further analysis.

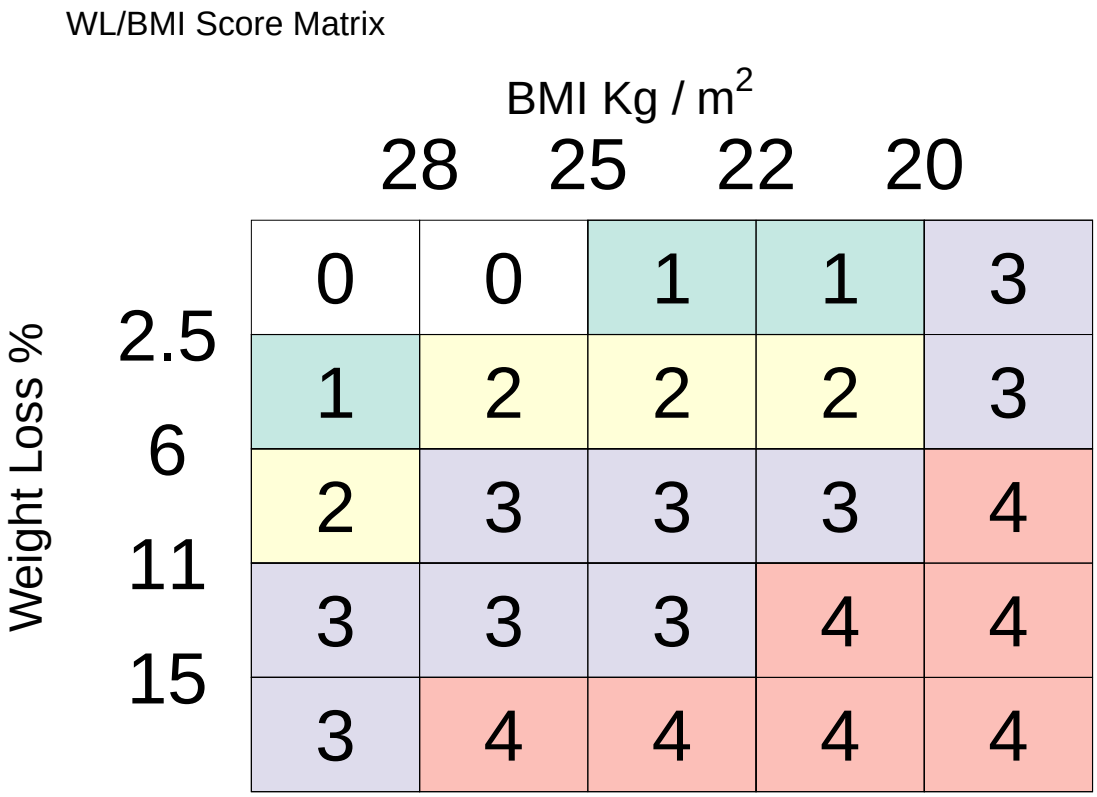



Figure 3.1: BMI/weight-loss Score Matrix

## 3.8 Sample collection

Under general anaesthesia, at the time of planned surgery, tissue samples of approximately 1cm x 1cm x 1cm size were taken by the operating surgeon. This was from the rectus abdominis muscle at the time of laparotomy for the UGIC and AAA patients. The muscle samples from the LDN group were collected at the time of laparoscopic port insertion (either single-incision laparoscopic surgical port or hand-assistance port), also from the rectus abdominis muscle.

Skeletal muscle tissue samples were cleaned of obvious adipose and connective tissue and then divided into aliquots: 4 were paced into cryovials and then snap-frozen in liquid nitrogen. 2 further aliquots were aligned in the line of their fibres in Optimum Cutting Temperature (OCT) compound before being snap-frozen in isopentane which had been cooled in liquid nitrogen. All of these muscle samples were then stored at -80 degrees C until analysis.

Blood was taken in 2 x serum gel tubes and 1 x ethylene dia-amine tetra-acetic acid (EDTA) tube during anaesthetic induction or via an in-dwelling arterial catheter intra-operatively. The samples were centrifuged at 1500G for 10 mins then the serum was divided into 2ml aliquots and snap-frozen before being stored at -80C. The EDTA cell pellet was snap-frozen and stored at -80C however the serum gel cell pellet was discarded due to gel beads mixing through the cells.

## 3.9 Biochemical analysis

### 3.9.1 Skeletal Muscle Protein Content

The skeletal muscle biopsies were pulverized and weighed using an analytical balance (Mettler Toledo), then lysed with Phosphosafe Extraction Reagent (Merck Millipore, Billerica, Mass., USA) before being homogenized and centrifuged. The supernatant was analysed for total soluble protein (including myofibrillar and cystosolic) content using commercially available BCA protein assay kits (Pierce Biotechnology, Thermo Fisher Scientific, Rockford, IL).

This work was carried out by Dr Carsten Jacobi in Basel, who describes the process in depth as below:

"Patient samples were first powdered (covaris protocol) then powder was weight out on a precision scale and lysed in phosphosafe buffer using precellys protocol and centrifuged. Lysate Supernatant was subjected to protein concentration measurement with BCA and 10µg of total protein was loaded per PolyAcrylamide Gel

Electrophoresis (PAGE) slot for Western Blot and another PAGE gel for coomassie staining of each sample in order to normalize the Western Blot signal on the proteome (imaging of the coomassie stained PAGE and Western Blot and evaluation using Fiji)." 1. The protein determination by the BCA method, here technical replicates (n=3) were used

2. In addition as an orthogonal verification, samples were separated by PAGE and all proteins visualized by coomassie staining. The "whole protein stain" was scanned and quantified using Fiji and determine the optical density of the complete stain.

### 3.9.2 Skeletal Muscle Fibre Cross-Sectional Area

Muscle fibre cross-sectional area was ascertained as follows: Initially, a microtome was used to thinly slice the OCT-embedded samples. The frozen muscle sections were co-stained for laminin (L9393, Sigma-Aldrich, Buchs, Switzerland) and myosin heavy chain type I or IIa to distinguish each fibre type (BA-D5 for type I, SC-71 for type IIa). The paraffin sections were stained for phospho-STAT3 (D3A7, Cell Signaling Technologies, Danvers, MA, USA) with a Ventana discovery XT (Roche group, Tucson, USA) to identify cells by presence of nuclei. Images of the entire tissue section were acquired using a VS120 slide scanner (Olympus Corporation, Tokyo, Japan). The distribution of myosin heavy chain fibre types, the cross section area of the individual fibres in the section, and the phospho-STAT3 positive nuclei and staining density were analysed using the proprietary image analysis platform ASTORIA (Automated Stored Image Analysis) developed by Novartis/Preclinical Safety.
This work was carried out by Dr Carsten Jacobi in Basel.

## 3.10 Statistical analysis

Statistical analysis was performed in R [168]. Quality of life data was analysed with QoLR [163]. Tables were constructed using FinalFit [169]. After graphical analysis for normality, parametric and non-parametric statistical tests, including two-tailed t-tests were conducted where appropriate, as were Chi-Squared tests, and linear regression. A p value of $<0.05$ was taken to indicate statistical significance, and differences in means were assessed individually to ascertain clinical significance. As this was an exploratory analysis, corrections for multiple testing were not applied. For the QLQ-C30+OG25 scores, a difference of $>10$ points was taken to indicate clinically relevant differences in symptomatic and functional domain scores. Multivariable analysis was performed utilising the olsrr package [170] in R. The data were tested for normality, and a series of candidate

variables were chosen based on their univariate relationship with the variable under test. Stepwise regression was then undertaken, with $p < 0.1$ used as the threshold for inclusion in the model, and $p > 0.3$ used as the threshold for exclusion from the model. Following model production residual diagnostics were performed to ensure the model was robust, including qq-plotting, Shapiro-Wilk test, Kolmogorov-Smirnov test, Cramer-von Mises test, and Anderson-Darling test. The Breusch Pagan test was used to assess for heteroskedasticity. Models passing these tests were reported using FinalFit [169], and those failing were discarded.

## 3.11 Missing data

An initial missing data anlaysis was conducted using the multiple options available in the FinalFit package [169], as described in the relevant technical manual. The outcomes from this did reveal missing data, and the patterns of this.

During the initial phase of the study, permissions for use of various questionnaires were awaited prior to use. Accordingly, there are a number of cases in which the participants did not complete various questionnaires. Whilst this may not be strictly a "missing completely at random" case, there is no obvious or explicable relationship between the missing data and other observed data. It could also be argued that the failure to offer questionnaires to initially recruited patients could reflect a degree of sampling bias. Additionally, and when considering the types of answers generated by the questionnaires when applied to participants completing them, it was felt that imputation may not be appropriate and accordingly analysis was conducted using list-wise deletion. This reduces the number of cases to analyse, but does not introduce other potential sources of error. Similarly, for objective physical measurements such as gait speed, when not measured in the clinical environment it was felt more suitable to analyse complete cases rather than impute for missing data.

With regards to the biochemical data, due to constraints from the external laboratory analysing the physical samples, not all patients had all of their samples analysed. This is particularly notable for the muscle fibre size cross-sectional area. Again, and as this is a relatively new field, it was not felt that attempting to impute data would be appropriate and so complete case analyses were performed.

Overall, the missingness of any particular data points was not felt to be in any way related to any observed data values. Accordingly the missing data were treated as *missing completely at random*. As this was an observational study looking to ascertain the presence or absence of a relationship between patient phenotype and any of the outcomes as discussed in each individual chapter, it follows that each variable has potential as an explanatory variable, and so imputation should not be used. As mentioned above tests were conducted

using complete case analyses.

# Chapter 4

# Demographics of Recruited Patients

Between December 2015 and August 2017 194 patients were recruited, of 3 broad types (n, Male(%)): Live donor nephrectomy (LDN) (53, 24(45.3%)); Vascular aortic (AAA) (52, 44(84.6%)); and Upper GI Cancer (UGIC) (89, 62(69.7%)) patients. Mean age overall was 63 years (range 25-90).
As discussed in the Methods chapter, data were plotted to assess normality, and parametric and non-parametric statistical tests were then applied accordingly.

## 4.1 LDN Patients

Within this patient group, mean age was 51.7 years (25-77). A further breakdown of other collected phenotypic data by sex is presented in Table 4.1. Males were taller (176.9 v 162.4cm, $p < 0.001$), heavier (79.9 v 67.6Kg, $p < 0.001$), and had greater SMI (51.5 v 42$cm^2/m^2$, $p < 0.001$) than females. There was no statistically significant difference in weight loss, gait speed, or rates of sarcopenia between the sexes.

Table 4.1: LDN Patient Demographics

| Sex | N (total) | N (missing) | | All | Female | Male | p |
|---|---|---|---|---|---|---|---|
| Age | 53 | 0 | Mean (SD) | 51.7 (12.2) | 50.8 (15.0) | 52.4 (9.5) | 0.627 |
| Height (cm) | 53 | 0 | Mean (SD) | 168.9 (10.0) | 176.9 (6.7) | 162.4 (7.2) | <0.001 |
| Weight | 53 | 0 | Mean (SD) | 73.2 (13.0) | 79.9 (12.2) | 67.6 (10.9) | <0.001 |
| Weight Loss (%) | 53 | 0 | Mean (SD) | 3.7 (10.7) | 1.6 (3.8) | 5.4 (13.9) | 0.196 |
| BMI | 53 | 0 | Mean (SD) | 25.6 (3.1) | 25.6 (3.2) | 25.6 (3.1) | 0.971 |
| SMI | 53 | 0 | Mean (SD) | 46.3 (6.7) | 51.5 (5.5) | 42.0 (3.9) | <0.001 |
| Sarcopenia | 53 | 0 | No | 32 (60.4) | 15 (62.5) | 17 (58.6) | 0.996 |
| | | | Yes | 21 (39.6) | 9 (37.5) | 12 (41.4) | |
| SMD | 53 | 0 | Mean (SD) | 42.1 (7.2) | 43.0 (7.6) | 41.3 (6.9) | 0.414 |
| Gait Speed (m/s) | 41 | 12 | Mean (SD) | 1.6 (0.3) | 1.6 (0.4) | 1.7 (0.3) | 0.334 |
| Cachexia | 53 | 0 | No Cachexia | 40 (75.5) | 18 (75.0) | 22 (75.9) | 1.000 |
| | | | Cachexia | 13 (24.5) | 6 (25.0) | 7 (24.1) | |

*Note:*
NaN indicates missing values, NA indicates the test was unable to be run. Tests used include Wilcoxon-Kruskal-Wallis test for continuous variables, Pearson chi-square test for categorical variables, and the likelihood ratio chi-square test from the proportional odds model for ordinal variables.

Table 4.2: AAA Patient Demographics

| Sex | N (total) | N (missing) | | All | Female | Male | p |
|---|---|---|---|---|---|---|---|
| Age | 52 | 0 | Mean (SD) | 66.5 (10.4) | 65.1 (10.1) | 74.2 (8.8) | 0.021 |
| Height (cm) | 52 | 0 | Mean (SD) | 173.9 (9.8) | 176.4 (8.1) | 160.1 (6.2) | <0.001 |
| Weight | 52 | 0 | Mean (SD) | 83.6 (21.7) | 88.0 (20.0) | 58.9 (12.9) | <0.001 |
| Weight Loss (%) | 52 | 0 | Mean (SD) | 1.4 (6.1) | 1.3 (6.3) | 2.0 (4.7) | 0.751 |
| BMI | 52 | 0 | Mean (SD) | 27.2 (5.1) | 28.0 (5.0) | 22.9 (4.1) | 0.008 |
| SMI | 52 | 0 | Mean (SD) | 47.7 (8.7) | 49.0 (8.3) | 40.4 (7.8) | 0.009 |
| Sarcopenia | 52 | 0 | No | 23 (44.2) | 19 (43.2) | 4 (50.0) | 1.000 |
| | | | Yes | 29 (55.8) | 25 (56.8) | 4 (50.0) | |
| SMD | 52 | 0 | Mean (SD) | 33.3 (9.8) | 33.2 (10.5) | 33.8 (4.5) | 0.859 |
| Gait Speed (m/s) | 38 | 14 | Mean (SD) | 1.3 (0.4) | 1.3 (0.3) | 1.6 (0.8) | 0.158 |
| Frailty Category | 35 | 17 | No Frailty | 30 (57.7) | 29 (65.9) | 1 (12.5) | 0.208 |
| | | | Pre-frail | 4 (7.7) | 3 (6.8) | 1 (12.5) | |
| | | | Frail | 1 (1.9) | 1 (2.3) | | |
| | | | (Missing) | 17 (32.7) | 11 (25.0) | 6 (75.0) | |
| Cachexia | 52 | 0 | No Cachexia | 41 (78.8) | 35 (79.5) | 6 (75.0) | 1.000 |
| | | | Cachexia | 11 (21.2) | 9 (20.5) | 2 (25.0) | |

*Note:*
NaN indicates missing values, NA indicates the test was unable to be run. Tests used include Wilcoxon-Kruskal-Wallis test for continuous variables, Pearson chi-square test for categorical variables, and the likelihood ratio chi-square test from the proportional odds model for ordinal variables.

## 4.2 AAA Patients

Within this patient type, mean age was 66.5 years (29-90). Again, more detailed phenotypic data is presented although it should be noted that the number of females in this patient type is low. The data are presented in Table 4.2.

In the AAA patient type, males were younger (65.1 v 74.2 years, $p = 0.03$), taller (176.4 v 160.1cm, $p < 0.001$), heavier (88 v 58.9Kg, $p < 0.001$), and had higher SMI (49.0 v 40.4$cm^2/m^2$) than females. The other statistically significant difference noted is calculated from weight and height.

## 4.3 UGIC Patients

In the UGIC patient type, mean age was 67.6 (47-87). These patients consisted of gastric (n=18); oesophageal adenocarcinoma (n=45), oesophageal squamous carcinoma (n=10), and pancreatic (n=12) cancer patients. 1 patient had a sarcomatoid oesophageal cancer, and 3 patients did not have their tumour type recorded This was due to their cases being investigated in other health boards and no record of pathological investigation available in the study centre. Clinical staging information is shown in Table 4.3; and pathological staging is shown in Table 4.4 where available. It is to be noted that some patients were diagnosed with their tumours at their local institutions and these reports frequently do not code sufficient information to allow a clinical tumour, nodal, and metastatic staging (cTNM). Additionally, where palliative operations were performed, the tumours were not resected and thus pathological staging (pTNM) is not available. These cases are marked *"Unstaged"*.

Demographic data are shown in Table 4.5. This reveals that male UGIC patients were taller (176.6 v 159.5cm, $p < 0.001$), heavier (83.8 v 68.5Kg, $p < 0.001$), had higher SMI (48.7 v 42.9$cm^2/m^2$, $p = 0.002$), and had a higher gait speed (1.5 v 1.3m/s, $p = 0.011$). There were no statistically significant differences in rates of sarcopenia or cachexia between sexes.

Table 4.3: Clinical Stage by Cancer Type and by Sex

| Cancer Type | Sex | Clinical Stage | n |
|---|---|---|---|
| Gastric | Male | 1 | 3 |
| | | 3 | 6 |
| | Female | 1 | 4 |
| | | 2a | 1 |
| | | 2b | 3 |
| | | 3 | 1 |
| Oesophageal Adeno | Male | 1 | 4 |
| | | 2b | 1 |
| | | 3 | 20 |
| | | 4 | 13 |
| | | Unstaged | 1 |
| | Female | 1 | 1 |
| | | 3 | 3 |
| | | 4 | 2 |
| Oesophageal Squamous | Male | 1 | 1 |
| | | 2 | 1 |
| | | 3 | 3 |
| | Female | 1 | 2 |
| | | 2 | 1 |
| | | 3 | 2 |
| | | 1a | 2 |
| | | 1b | 2 |

Table 4.3: Clinical Stage by Cancer Type and by Sex *(continued)*

| Cancer Type | Sex | Clinical Stage | n |
|---|---|---|---|
| Pancreatic | Male | 2a | 1 |
| | | 2b | 1 |
| | Female | 1a | 1 |
| | | 1b | 1 |
| | | 2a | 2 |
| | | 2b | 1 |
| | | Unstaged | 1 |

Table 4.4: Pathological Stage by Cancer Type and by Sex

| Cancer Type | Sex | Pathological Stage | n |
|---|---|---|---|
| Gastric | Male | 1a | 2 |
| | | 1b | 1 |
| | | 2a | 2 |
| | | 2b | 1 |
| | | 3a | 1 |
| | | 3b | 1 |
| | | Unstaged | 1 |
| | Female | 0 | 1 |
| | | 1a | 2 |
| | | 1b | 1 |
| | | 2a | 2 |
| | | 2b | 1 |
| | | 3a | 1 |
| | | 3b | 1 |
| | Male | 0 | 1 |
| | | 1b | 6 |
| | | 2a | 2 |
| | | 2b | 1 |
| | | 3a | 8 |
| | | 3b | 13 |
| | | 4 | 7 |
| | | Unstaged | 1 |

Table 4.4: Pathological Stage by Cancer Type and by Sex *(continued)*

| Oesophageal Adeno Cancer Type | Sex | Pathological Stage | n |
|---|---|---|---|
| | | 1a | 1 |
| | | 3a | 2 |
| | Female | 4 | 2 |
| | | Unstaged | 1 |
| | | 1a | 1 |
| | | 1b | 1 |
| | Male | 2 | 1 |
| | | 3b | 2 |
| Oesophageal Squamous | | 1a | 1 |
| | Female | 3a | 1 |
| | | 3b | 3 |
| | | 2a | 1 |
| | Male | 3b | 4 |
| | | Unstaged | 1 |
| Pancreatic | | 2a | 2 |
| | Female | 3b | 2 |
| | | Unstaged | 2 |

Table 4.5: UGIC Patient Demographics

| Sex | N (total) | N (missing) | | All | Female | Male | p |
|---|---|---|---|---|---|---|---|
| Age | 89 | 0 | Mean (SD) | 67.7 (9.3) | 67.3 (8.7) | 68.6 (10.7) | 0.536 |
| Height (cm) | 89 | 0 | Mean (SD) | 171.4 (9.9) | 176.6 (6.2) | 159.5 (5.5) | <0.001 |
| Weight | 89 | 0 | Mean (SD) | 79.1 (18.5) | 83.8 (16.7) | 68.5 (18.2) | <0.001 |
| Weight Loss (%) | 89 | 0 | Mean (SD) | 6.6 (7.6) | 7.0 (7.2) | 5.6 (8.5) | 0.449 |
| BMI | 89 | 0 | Mean (SD) | 26.9 (5.8) | 26.9 (5.5) | 26.9 (6.3) | 0.986 |
| SMI | 89 | 0 | Mean (SD) | 47.0 (8.5) | 48.7 (8.7) | 42.9 (6.6) | 0.003 |
| Sarcopenia | 89 | 0 | No | 47 (52.8) | 30 (48.4) | 17 (63.0) | 0.301 |
| | | | Yes | 42 (47.2) | 32 (51.6) | 10 (37.0) | |
| SMD | 89 | 0 | Mean (SD) | 34.9 (7.9) | 34.7 (8.1) | 35.3 (7.4) | 0.746 |
| Gait Speed (m/s) | 58 | 31 | Mean (SD) | 1.4 (0.3) | 1.5 (0.3) | 1.3 (0.3) | 0.014 |
| Frailty Category | 53 | 36 | No Frailty | 46 (51.7) | 31 (50.0) | 15 (55.6) | 0.916 |
| | | | Pre-frail | 7 (7.9) | 4 (6.5) | 3 (11.1) | |
| | | | (Missing) | 36 (40.4) | 27 (43.5) | 9 (33.3) | |
| Cachexia | 89 | 0 | No Cachexia | 36 (40.4) | 23 (37.1) | 13 (48.1) | 0.458 |
| | | | Cachexia | 53 (59.6) | 39 (62.9) | 14 (51.9) | |

*Note:*
NaN indicates missing values, NA indicates the test was unable to be run. Tests used include Wilcoxon-Kruskal-Wallis test for continuous variables, Pearson chi-square test for categorical variables, and the likelihood ratio chi-square test from the proportional odds model for ordinal variables.

## 4.4 Demographic Comparisons Between Patient Types

Demographic assessments were carried out comparing each group with the others in a stepped fashion, to ensure no comparisons were left undone. These are shown in Table 4.6 for LDN vs AAA patients, in Table 4.7 for AAA vs UGIC patients, and in Table 4.8 for LDN vs UGIC patients.

A subsequent "whole-cohort" comparison is included to allow easier comparison between all groups.

### 4.4.1 Demographic Comparison Between LDN and AAA Patients

This comparison was performed to assess the similarity or otherwise of the non-cancer patient types. This reveals that the LDN patients were less predominantly male (35.3 v 64.7%, $p < 0.001$); younger (51.7 v 66.5 years, $p < 0.001$); shorter of stature (168.9 v 173.9cm, $p = 0.016$); lighter (73.2 v 83.6Kg, $p = 0.012$); had a higher SMD (42.1 v 33.1, $p < 0.001$); had a higher gait speed (1.6 v 1.4m/s, $p < 0.001$); and had a greater proportion of patients in the *"No Frailty"* category (56.5 v 43.5%, $p = 0.05$). There were no significant differences in BMI; SMI; weight loss; or cachexia prevalence. This is to be expected when comparing a selected healthy patient type with a type known to have long-standing disease.

Table 4.6: LDN vs AAA Patient Demographics

| Sex | N (total) | N (missing) | | All | Female | Male | p |
|---|---|---|---|---|---|---|---|
| Sex | 105 | 0 | Male | 68 (64.8) | 24 (45.3) | 44 (84.6) | <0.001 |
| | | | Female | 37 (35.2) | 29 (54.7) | 8 (15.4) | |
| Age | 105 | 0 | Mean (SD) | 59.0 (13.5) | 51.7 (12.2) | 66.5 (10.4) | <0.001 |
| Height (cm) | 105 | 0 | Mean (SD) | 171.4 (10.2) | 168.9 (10.0) | 173.9 (9.8) | 0.012 |
| Weight | 105 | 0 | Mean (SD) | 78.3 (18.5) | 73.2 (13.0) | 83.6 (21.7) | 0.004 |
| Weight Loss (%) | 105 | 0 | Mean (SD) | 2.5 (8.7) | 3.7 (10.7) | 1.4 (6.1) | 0.182 |
| BMI | 105 | 0 | Mean (SD) | 26.4 (4.3) | 25.6 (3.1) | 27.2 (5.1) | 0.044 |
| SMI | 105 | 0 | Mean (SD) | 47.0 (7.7) | 46.3 (6.7) | 47.7 (8.7) | 0.367 |
| Sarcopenia | 105 | 0 | No | 55 (52.4) | 32 (60.4) | 23 (44.2) | 0.144 |
| | | | Yes | 50 (47.6) | 21 (39.6) | 29 (55.8) | |
| SMD | 105 | 0 | Mean (SD) | 37.7 (9.6) | 42.1 (7.2) | 33.3 (9.8) | <0.001 |
| Gait Speed (m/s) | 79 | 26 | Mean (SD) | 1.5 (0.4) | 1.6 (0.3) | 1.3 (0.4) | <0.001 |
| Frailty Category | 74 | 31 | No Frailty | 69 (65.7) | 39 (73.6) | 30 (57.7) | 0.050 |
| | | | (Missing) | 31 (29.5) | 14 (26.4) | 17 (32.7) | |
| | | | Pre-frail | 4 (3.8) | | 4 (7.7) | |
| | | | Frail | 1 (1.0) | | 1 (1.9) | |
| Cachexia | 105 | 0 | No Cachexia | 81 (77.1) | 40 (75.5) | 41 (78.8) | 0.858 |
| | | | Cachexia | 24 (22.9) | 13 (24.5) | 11 (21.2) | |

*Note:*
NaN indicates missing values, NA indicates the test was unable to be run. Tests used include Wilcoxon-Kruskal-Wallis test for continuous variables, Pearson chi-square test for categorical variables, and the likelihood ratio chi-square test from the proportional odds model for ordinal variables.

Table 4.7: UGIC vs AAA Patient Demographics

| Sex | N (total) | N (missing) | | All | Female | Male | p |
|---|---|---|---|---|---|---|---|
| Sex | 141 | 0 | Male | 106 (75.2) | 44 (84.6) | 62 (69.7) | 0.075 |
| | | | Female | 35 (24.8) | 8 (15.4) | 27 (30.3) | |
| Age | 141 | 0 | Mean (SD) | 67.2 (9.7) | 66.5 (10.4) | 67.7 (9.3) | 0.508 |
| Height (cm) | 141 | 0 | Mean (SD) | 172.3 (9.9) | 173.9 (9.8) | 171.4 (9.9) | 0.151 |
| Weight | 141 | 0 | Mean (SD) | 80.8 (19.8) | 83.6 (21.7) | 79.1 (18.5) | 0.202 |
| Weight Loss (%) | 141 | 0 | Mean (SD) | 4.6 (7.5) | 1.4 (6.1) | 6.6 (7.6) | <0.001 |
| BMI | 141 | 0 | Mean (SD) | 27.0 (5.5) | 27.2 (5.1) | 26.9 (5.8) | 0.726 |
| SMI | 141 | 0 | Mean (SD) | 47.2 (8.6) | 47.7 (8.7) | 47.0 (8.5) | 0.639 |
| Sarcopenia | 141 | 0 | No | 70 (49.6) | 23 (44.2) | 47 (52.8) | 0.419 |
| | | | Yes | 71 (50.4) | 29 (55.8) | 42 (47.2) | |
| SMD | 141 | 0 | Mean (SD) | 34.3 (8.6) | 33.3 (9.8) | 34.9 (7.9) | 0.278 |
| Gait Speed (m/s) | 96 | 45 | Mean (SD) | 1.4 (0.3) | 1.3 (0.4) | 1.4 (0.3) | 0.076 |
| Frailty Category | 88 | 53 | No Frailty | 76 (53.9) | 30 (57.7) | 46 (51.7) | 0.456 |
| | | | Pre-frail | 11 (7.8) | 4 (7.7) | 7 (7.9) | |
| | | | Frail | 1 (0.7) | 1 (1.9) | | |
| | | | (Missing) | 53 (37.6) | 17 (32.7) | 36 (40.4) | |
| Cachexia | 141 | 0 | No Cachexia | 77 (54.6) | 41 (78.8) | 36 (40.4) | <0.001 |
| | | | Cachexia | 64 (45.4) | 11 (21.2) | 53 (59.6) | |

*Note:*
NaN indicates missing values, NA indicates the test was unable to be run. Tests used include Wilcoxon-Kruskal-Wallis test for continuous variables, Pearson chi-square test for categorical variables, and the likelihood ratio chi-square test from the proportional odds model for ordinal variables.

### 4.4.2 Demographic Comparison Between UGIC and AAA Patients

The AAA and UGIC patients were compared to ascertain how closely the patients of each type matched. The data reveal that the patients of each type are more closely matched than the AAA with the LDN patients. Comparing UGIC to AAA patients, the data show that the UGIC patients had more weight loss (6.6 v 1.4%, $p < 0.001$); had a faster gait speed (1.4 v 1.4m/s, $p = 0.045$), and had fewer patients in the *"No cachexia"* category (46.8 v 53.2%, $p < 0.001$). Other demographic differences did not reach significance, including age; height; weight; SMI; and SMD. This could partly be expected as both the AAA and UGIC patients are older and may thus have similar co-morbidities, but the lack of difference in SMI and SMD is slightly surprising.

Table 4.8: UGIC vs LDN Patient Demographics

| Sex | N (total) | N (missing) | | All | Female | Male | p |
|---|---|---|---|---|---|---|---|
| Sex | 142 | 0 | Male | 86 (60.6) | 24 (45.3) | 62 (69.7) | 0.007 |
| | | | Female | 56 (39.4) | 29 (54.7) | 27 (30.3) | |
| Age | 142 | 0 | Mean (SD) | 61.7 (13.0) | 51.7 (12.2) | 67.7 (9.3) | <0.001 |
| Height (cm) | 142 | 0 | Mean (SD) | 170.5 (10.0) | 168.9 (10.0) | 171.4 (9.9) | 0.156 |
| Weight | 142 | 0 | Mean (SD) | 76.9 (16.8) | 73.2 (13.0) | 79.1 (18.5) | 0.041 |
| Weight Loss (%) | 142 | 0 | Mean (SD) | 5.5 (8.9) | 3.7 (10.7) | 6.6 (7.6) | 0.062 |
| BMI | 142 | 0 | Mean (SD) | 26.4 (5.0) | 25.6 (3.1) | 26.9 (5.8) | 0.119 |
| SMI | 142 | 0 | Mean (SD) | 46.7 (7.9) | 46.3 (6.7) | 47.0 (8.5) | 0.627 |
| Sarcopenia | 142 | 0 | No | 79 (55.6) | 32 (60.4) | 47 (52.8) | 0.482 |
| | | | Yes | 63 (44.4) | 21 (39.6) | 42 (47.2) | |
| SMD | 142 | 0 | Mean (SD) | 37.6 (8.4) | 42.1 (7.2) | 34.9 (7.9) | <0.001 |
| Gait Speed (m/s) | 99 | 43 | Mean (SD) | 1.5 (0.3) | 1.6 (0.3) | 1.4 (0.3) | 0.001 |
| Frailty Category | 92 | 50 | No Frailty | 85 (59.9) | 39 (73.6) | 46 (51.7) | 0.050 |
| | | | (Missing) | 50 (35.2) | 14 (26.4) | 36 (40.4) | |
| | | | Pre-frail | 7 (4.9) | | 7 (7.9) | |
| Cachexia | 142 | 0 | No Cachexia | 76 (53.5) | 40 (75.5) | 36 (40.4) | <0.001 |
| | | | Cachexia | 66 (46.5) | 13 (24.5) | 53 (59.6) | |

*Note:*
NaN indicates missing values, NA indicates the test was unable to be run. Tests used include Wilcoxon-Kruskal-Wallis test for continuous variables, Pearson chi-square test for categorical variables, and the likelihood ratio chi-square test from the proportional odds model for ordinal variables.

### 4.4.3 Demographic Differences Between UGIC and LDN Patient Types

A comparison between UGIC and LDN patients could be expected to reveal the greatest differences, and some of these do appear in Table 4.8. The comparison reveals that the UGIC patient type was more predominantly male (n= 62 v 24, $p = 0.004$); older (67.7 v 51.7 years, $p < 0.001$); had greater weight loss (6.6 v 3.7Kg, $p < 0.001$); lower SMD (34.9 v 42.1 HU, $p < 0.001$); lower gait speed (1.4 v 1.6m/s, $p = 0.005$); and were more likely to be cachectic (n=53 v 13, $p < 0.001$). This is not overly surprising. What is slightly surprising, however, is the absence of significant difference in SMI. Although both patient types do not have statistically significant differences in height or weight, the greater weight loss in UGIC patients could be expected to be reflected in a reduced SMI. This is not the case, and it is difficult to reconcile this absence of difference with the international consensus definition which includes the phrase *"ongoing loss of skeletal muscle mass"*. That the weight loss is not reflected in reduced SMI could point to a higher starting SMI, loss of non-muscular tissue, or a need for an alternative definition of cachexia.

### 4.4.4 Combined demographics

Having examined all combinations of groups to ensure no important comparisons were missed, ANOVA analysis was undertaken to compare all groups together, and to ensure values obtained for each group were easily compared to those obtained from the entire cohort overall. This allowed an examination of how far above or below the whole cohort mean each group was for each variable, and also allowed easy assessment of categorical variables between groups. This analysis is shown in Table 4.9.

As can be seen, the differences between patient groups noted in the comparisons tabulated above are demonstrated. Of particular note are the differences in age, (LDN 51.7 v AAA 66.5 v UGIC 67.7 $p<0.001$), weight loss percent (LDN 3.7 v AAA 1.4 v UGIC 6.6 $p=0.001$), SMD (LDN 42.1 v AAA 33.3 v UGIC 34.9 $p<0.001$), and gait speed (LDN 1.6 v AAA 1.3 v UGIC 1.4 $p<0.001$). American Society of Anaesthetists (ASA) grade also revealed a difference between groups in this analysis, with only LDN patients falling into ASA 1 and having no ASA 3 or ASA 4 patients in this group. Neither the AAA or UGIC groups had any ASA 1 patients, which might be expected given they have either vascular disease or cancer, and interestingly the AAA group had a higher percentage of ASA 3 patients than the UGIC group. Indeed, the AAA group was the only one to have patients scored as ASA 4.

It was particularly interesting to note that there was no statistically significant difference between the groups in BMI measurements, SMI measurements or in sarcopenia prevalence. This may be because the sarcopenia cut-points depend on SMI values and BMI categories to divide patients into subsets, however it remained clear that there was no difference in sarcopenia prevalence between groups. It is to be remembered that these patients are pre-operative candidates and so could be expected to have relatively maintained skeletal musculature compared to those at a later stage in their disease process.

Table 4.9: Whole-cohort Demographics

| Category | N (total) | N (missing) | | Live donor | Vascular | Cancer | All | p |
|---|---|---|---|---|---|---|---|---|
| Sex | 194 | 0 | Male | 24 (45.3) | 44 (84.6) | 62 (69.7) | 130 (67.0) | <0.001 |
| | | | Female | 29 (54.7) | 8 (15.4) | 27 (30.3) | 64 (33.0) | |
| Age | 194 | 0 | Mean (SD) | 51.7 (12.2) | 66.5 (10.4) | 67.7 (9.3) | 63.0 (12.5) | <0.001 |
| Height (cm) | 194 | 0 | Mean (SD) | 168.9 (10.0) | 173.9 (9.8) | 171.4 (9.9) | 171.4 (10.0) | 0.040 |
| Weight | 194 | 0 | Mean (SD) | 73.2 (13.0) | 83.6 (21.7) | 79.1 (18.5) | 78.7 (18.4) | 0.015 |
| Weight Loss (%) | 194 | 0 | Mean (SD) | 3.7 (10.7) | 1.4 (6.1) | 6.6 (7.6) | 4.4 (8.5) | 0.001 |
| BMI | 194 | 0 | Mean (SD) | 25.6 (3.1) | 27.2 (5.1) | 26.9 (5.8) | 26.6 (5.0) | 0.178 |
| SMI | 194 | 0 | Mean (SD) | 46.3 (6.7) | 47.7 (8.7) | 47.0 (8.5) | 47.0 (8.1) | 0.688 |
| SMD | 194 | 0 | Mean (SD) | 42.1 (7.2) | 33.3 (9.8) | 34.9 (7.9) | 36.4 (9.0) | <0.001 |
| Gait Speed (m/s) | 137 | 57 | Mean (SD) | 1.6 (0.3) | 1.3 (0.4) | 1.4 (0.3) | 1.5 (0.4) | <0.001 |
| ASA | 187 | 7 | 1 | 42 (79.2) | | | 42 (21.6) | <0.001 |
| | | | 2 | 11 (20.8) | 3 (5.8) | 50 (56.2) | 64 (33.0) | |
| | | | 3 | | 44 (84.6) | 35 (39.3) | 79 (40.7) | |
| | | | 4 | | 2 (3.8) | | 2 (1.0) | |
| | | | (Missing) | | 3 (5.8) | 4 (4.5) | 7 (3.6) | |
| Frailty Category | 127 | 67 | No Frailty | 39 (73.6) | 30 (57.7) | 46 (51.7) | 115 (59.3) | 0.087 |
| | | | (Missing) | 14 (26.4) | 17 (32.7) | 36 (40.4) | 67 (34.5) | |
| | | | Pre-frail | | 4 (7.7) | 7 (7.9) | 11 (5.7) | |
| | | | Frail | | 1 (1.9) | | 1 (0.5) | |
| Sarcopenia | 194 | 0 | No | 32 (60.4) | 23 (44.2) | 47 (52.8) | 102 (52.6) | 0.253 |
| | | | Yes | 21 (39.6) | 29 (55.8) | 42 (47.2) | 92 (47.4) | |
| Cachexia | 194 | 0 | No Cachexia | 40 (75.5) | 41 (78.8) | 36 (40.4) | 117 (60.3) | <0.001 |
| | | | Cachexia | 13 (24.5) | 11 (21.2) | 53 (59.6) | 77 (39.7) | |

*Note:*
NaN indicates missing values, NA indicates the test was unable to be run. Tests used include Wilcoxon-Kruskal-Wallis test for continuous variables, Pearson chi-square test for categorical variables, ANOVA for 3-way comparisons, and the likelihood ratio chi-square test from the proportional odds model for ordinal variables.

### 4.4.5 Missing data

It can be seen from the tables above, and in particular Table 4.9 that some measured variables have numbers of missing data. In particular, the variables relating to gait speed and to frailty have high numbers of missing data. This relates to delays in the provision of permissions to use certain questionnaires, and does potentially pose a problem for analysis. The treatment of missing data is discussed in the Methods chapter, but briefly a list-wise deletion technique was used to analyse complete cases only.

# Chapter 5

# CT Use In Defining Sarcopenia And Cachexia

As described in the Introduction chapter, the clinical relevance of CT-BCA has previously been investigated. Applying previously-described criteria to the current study population will allow assessment of their relevance to a preoperative surgical cancer cohort, and an assessment of how applicable these cancer-derived cut-points are in assessing selected *"healthy"* patients.

## 5.1 Hypothesis and research questions

### 5.1.1 Hypotheses

Published CT-BCA and cancer cachexia definitions apply well to recruited patients.

Cut-points derived from recruited patients match published values.

SMI values will be higher in healthy patients.

Cachexia will be seen in cancer patients only.

### 5.1.2 Research questions

- What are the means and differences in means of skeletal muscle CT-BCA variables in each group?
- What is the distribution of patients into *normal* and *low* CT-BCA categories if published criteria are applied to the current cohort?
- How well do cut-points derived from the literature apply to the current cohort?
- How well do cut-points derived from the current cohort relate to the published literature?
- How can cancer cachexia definitions be applied to non-cancer groups?

## 5.2 Methods for Assessing CT Use In Defining Sarcopenia And Cachexia

Between December 2015 and August 2017, 197 surgical patients were recruited to an observational study investigating the relationship between body composition, clinical phenotyping, and biochemistry. Patients were from one of 3 distinct groups: Upper GI cancer (UGIC), vascular aortic patients (AAA), and live kidney donors (LDN). Each patient was known to have had a pre-operative CT scan which was analysed at L3 level as described in Chapter 3, defining area measurements of skeletal muscle. These area measurements were indexed for patient height and BMI in a standard fashion [18]. CT phase corrections were carried out using equations published by Rollins *et al* [60]. Previously-published CT-BCA cut-points were applied [18]: normal BMI male SMI $<43 cm^2m^2$, high BMI male $<53 cm^2m^2$, normal BMI female $<41 cm^2m^2$, high BMI female $<41 cm^2m^2$; normal BMI male SMD <41 HU, high BMI male <33 HU, normal BMI female <41 HU, high BMI female <33 HU. Statistical analysis and data plotting were carried out using R [168]. New cohort-derived CT-BCA cut-points, in the absence of outcome data, were calculated by sex as described by Baumgartner *et al* [7] and by BMI as described by Martin *et al* [18]: specifically 2 standard deviations below the mean of a healthy population, in this case the LDN patients.

## 5.3 Results of the Assessment of CT Use In Defining Sarcopenia And Cachexia

### 5.3.1 Demographics for CT use in Sarcopenia and Cachexia

Basic demographics for the study cohort split by *Normal CT Muscularity* and *Low CT Muscularity* are presented in Table 5.1, and split by *Normal* and *Low* SMD are presented in Table 5.2.

These show that there was no significant difference across patient types, or in each sex by muscularity. They do, however, reveal that patients with low SMI were statistically significantly older than those with normal SMI (60 vs 66.3 years, $p < 0.001$). Patients with low SMI also had statistically significantly lower SMD (38.5 vs 34.1 HU, $p < 0.001$). When considering the SMD split, patients with low SMD were older (59 vs 69.6 years, $p < 0.001$) and had lower SMI (48.0 vs 45.3 $cm^2/m^2$). There were statistically significantly more UGIC patients in the normal SMD than the low SMD group (66 v 55, $p < 0.001$).

Table 5.1: Demographic Distribution by CT Muscularity

| Dependent: CT Muscularity | | Normal | Low muscularity | p |
|---|---|---|---|---|
| Group | Live donor | 32 (31.4) | 21 (22.8) | 0.253 |
| | Vascular | 23 (22.5) | 29 (31.5) | |
| | Cancer | 47 (46.1) | 42 (45.7) | |
| Sex | Male | 64 (62.7) | 66 (71.7) | 0.239 |
| | Female | 38 (37.3) | 26 (28.3) | |
| Age | Mean (SD) | 60.0 (11.9) | 66.3 (12.4) | <0.001 |
| BMI | Mean (SD) | 27.1 (5.3) | 26.1 (4.6) | 0.141 |
| Height (cm) | Mean (SD) | 169.7 (9.9) | 173.3 (9.9) | 0.013 |
| Weight (Kg) | Mean (SD) | 78.5 (18.3) | 78.9 (18.7) | 0.884 |
| SMI | Mean (SD) | 51.3 (7.5) | 42.2 (5.7) | <0.001 |
| SMD | Mean (SD) | 38.5 (8.0) | 34.1 (9.5) | 0.001 |

*Note:*
Tests used include Wilcoxon-Kruskal-Wallis test for continuous variables, Pearson chi-square test for categorical variables, and the likelihood ratio chi-square test from the proportional odds model for ordinal variables.

Table 5.2: Demographic Distribution by CT Radio-density

| Dependent: Muscle Radio-density | | Normal | Low Radio-density | p |
|---|---|---|---|---|
| Group | Live donor | 45 (32.8) | 8 (9.0) | <0.001 |
| | Vascular | 26 (19.0) | 26 (29.2) | |
| | Cancer | 66 (48.2) | 55 (61.8) | |
| Age (Years) | Mean (SD) | 59.0 (12.4) | 69.5 (8.6) | <0.001 |
| Sex | Male | 93 (67.9) | 63 (70.8) | 0.754 |
| | Female | 44 (32.1) | 26 (29.2) | |
| BMI | Mean (SD) | 26.6 (4.8) | 27.1 (5.6) | 0.458 |
| Height (cm) | Mean (SD) | 170.7 (9.0) | 172.8 (10.5) | 0.109 |
| Weight (Kg) | Mean (SD) | 77.8 (16.4) | 81.9 (21.6) | 0.105 |
| SMI | Mean (SD) | 48.0 (8.1) | 45.3 (8.2) | 0.014 |
| Protein Content | Mean (SD) | 123.6 (68.7) | 129.2 (78.9) | 0.653 |

*Note:*
Tests used include Wilcoxon-Kruskal-Wallis test for continuous variables, Pearson chi-square test for categorical variables, and the likelihood ratio chi-square test from the proportional odds model for ordinal variables.

Table 5.3: SMD comparison between groups

| Comparison | Sex | Difference | 1st Mean | 2nd Mean | Lower CI | Upper CI | p value |
|---|---|---|---|---|---|---|---|
| **UGIC vs LDN** | | | | | | | |
| | Male | 8.27 | 42.99 | 34.72 | 4.51 | 12.03 | 0.00006 |
| | Female | 6.03 | 41.34 | 35.32 | 2.16 | 9.89 | 0.00287 |
| **UGIC vs AAA** | | | | | | | |
| | Male | -1.57 | 33.16 | 34.72 | -5.33 | 2.20 | 0.40950 |
| | Female | -1.48 | 33.84 | 35.32 | -5.93 | 2.96 | 0.49392 |
| **LDN vs AAA** | | | | | | | |
| | Male | 9.84 | 42.99 | 33.16 | 5.40 | 14.27 | 0.00004 |
| | Female | 7.51 | 41.34 | 33.84 | 3.23 | 11.79 | 0.00172 |

### 5.3.2 Distribution of SMI and SMD

The histograms of SMI and SMD were assessed as a whole cohort, by sex, and by patient type within each sex. These are shown in Figure 5.1 and Figure 5.2 for SMI, and in Figure 5.3 and Figure 5.4 for SMD. Whilst there do appear to be differences between patient types, statistical testing did not reveal any significant differences in SMI in either male or female patients between types.

Statistical testing did, however, reveal significant differences in SMD between patient types, particularly between *"healthy"* and *"not healthy"* (LDN vs either UGIC or AAA) patients in both males and females. Interestingly, the difference in mean SMD was greater between LDN and AAA (M = 9.84, F = 7.51) than between LDN and UGIC (M = 8.27, F = 6.03). Also of interest is the finding that SMD does not statistically significantly differ between UGIC and AAA patients. This similarity between cancer and vascular patient CT-BCA has not been previously reported. These findings are shown in Table 5.3.

As previously discussed in Chapter 4, there are differences between these groups other than SMD. These possible confounding factors between groups were assessed to ascertain their relationship with SMD. Of these, only weight consistently showed a relationship with SMD across all patient groups. The relationship was seen in all patient groups, however, and appeared consistent on testing. Additionally, the differences in weight between groups was less than the standard deviation of weights in each group and accordingly it was not felt that these differences would account for the differences in SMD.

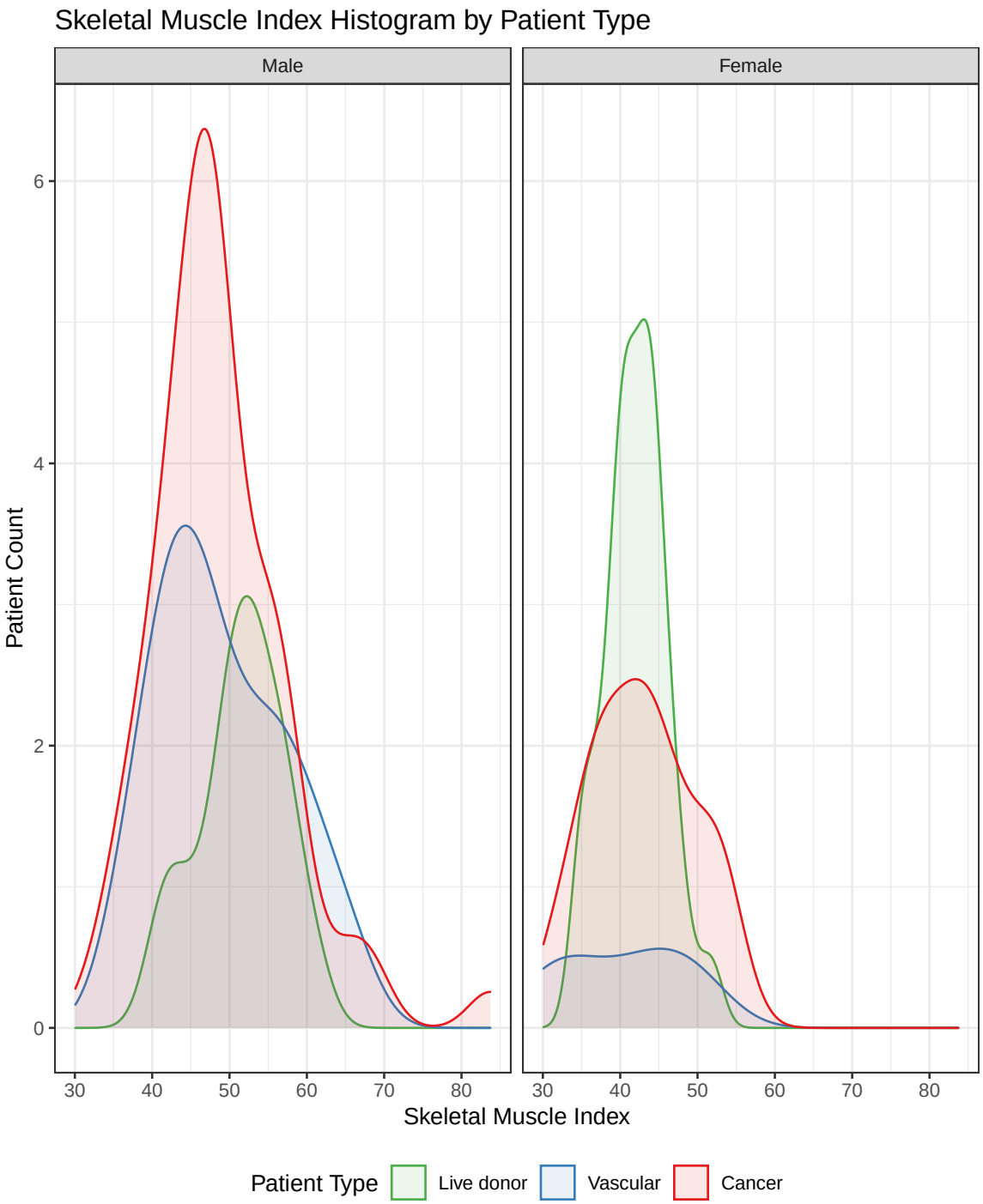



Figure 5.1: Patient Type SMI Density Curve

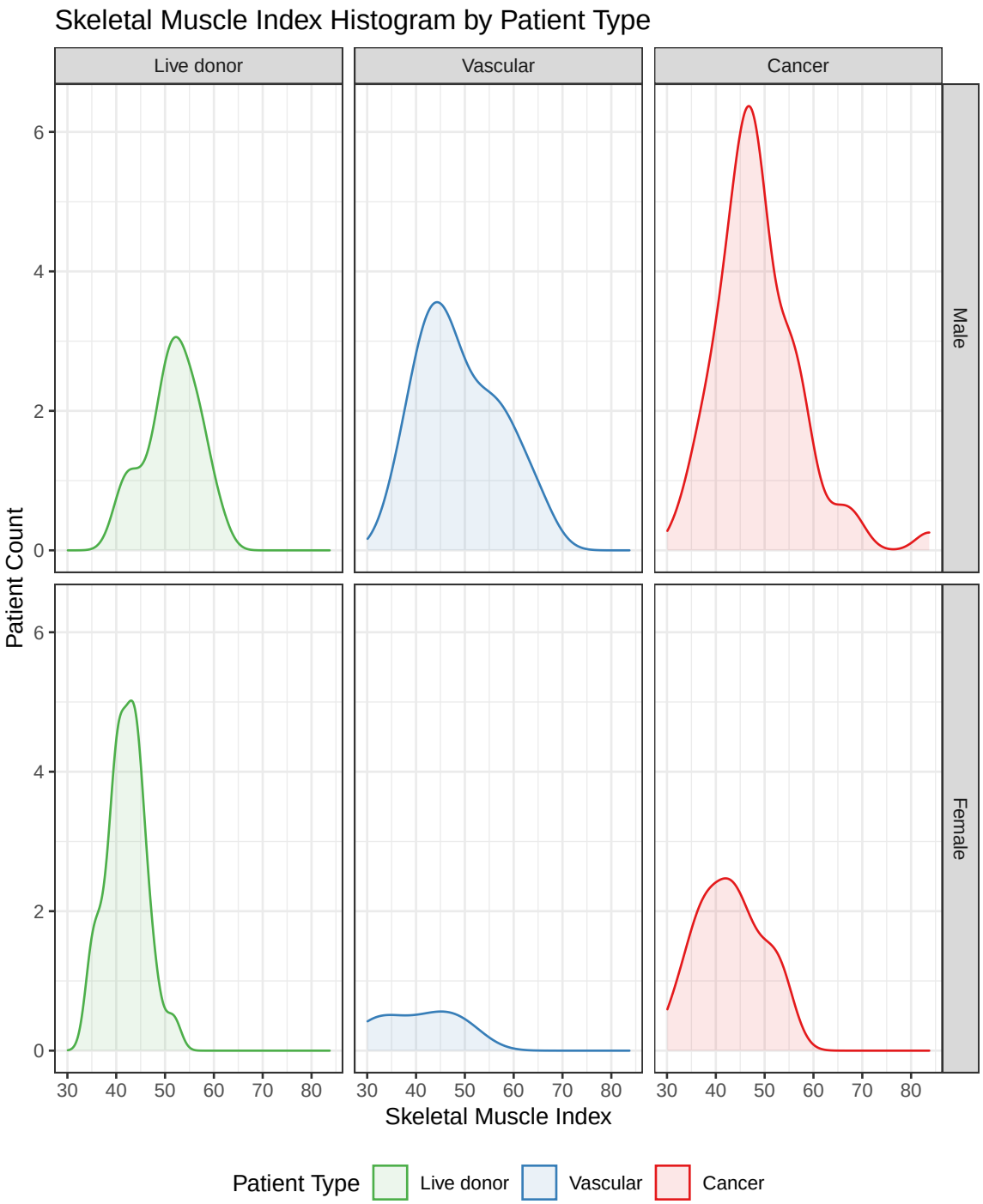



Figure 5.2: Patient SMI Density Curve Split by Type

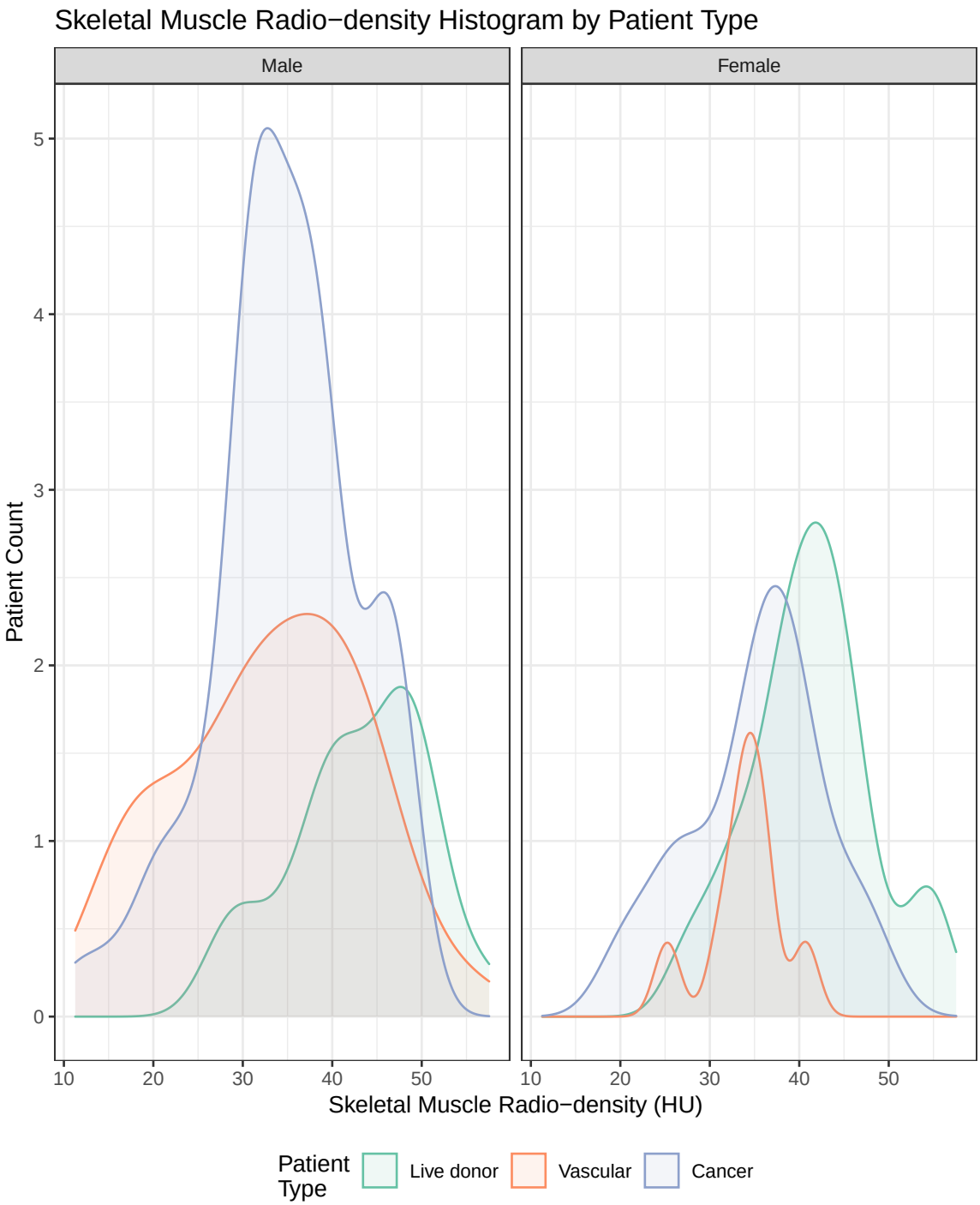



Figure 5.3: Patient Type SMD Density Curve

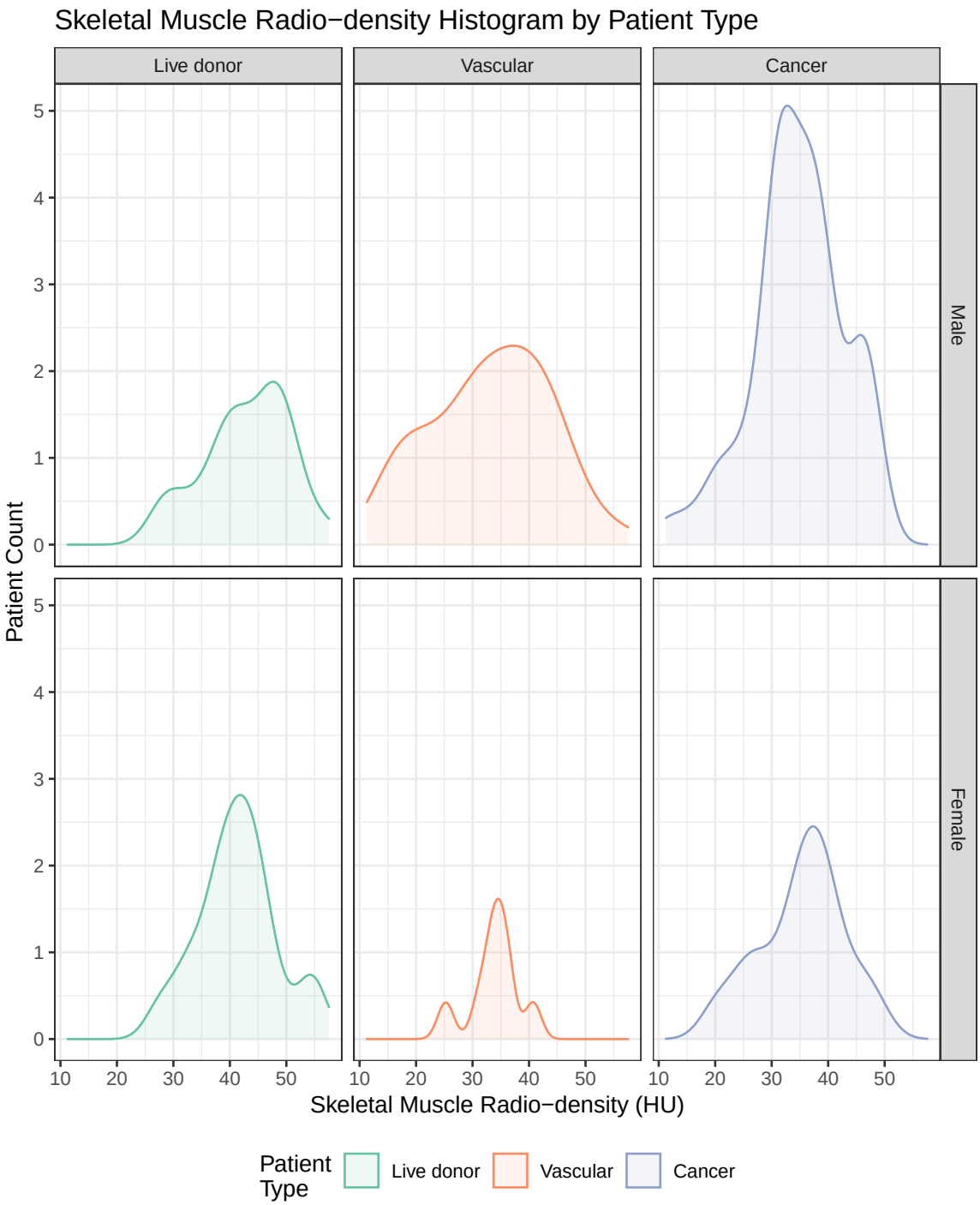



Figure 5.4: Patient SMD Density Curve Split by Type

Table 5.4: Number and Percentage of Patients of each Type by CT Muscularity

| Patient Type | Sex/BMI | Muscularity by SMI | n(%) per Type | Total per Type |
|---|---|---|---|---|
| **Live donor** | | | | |
| | Female | Normal | 17 (32.1%) | 53 |
| | Female | Low | 12 (22.6%) | 53 |
| | Low BMI Male | Normal | 6 (11.3%) | 53 |
| | Low BMI Male | Low | 3 (5.7%) | 53 |
| | High BMI Male | Normal | 9 (17%) | 53 |
| | High BMI Male | Low | 6 (11.3%) | 53 |
| **Vascular** | | | | |
| | Female | Normal | 4 (7.7%) | 52 |
| | Female | Low | 4 (7.7%) | 52 |
| | Low BMI Male | Normal | 6 (11.5%) | 52 |
| | Low BMI Male | Low | 6 (11.5%) | 52 |
| | High BMI Male | Normal | 13 (25%) | 52 |
| | High BMI Male | Low | 19 (36.5%) | 52 |
| **Cancer** | | | | |
| | Female | Normal | 17 (19.1%) | 89 |
| | Female | Low | 10 (11.2%) | 89 |
| | Low BMI Male | Normal | 15 (16.9%) | 89 |
| | Low BMI Male | Low | 6 (6.7%) | 89 |
| | High BMI Male | Normal | 15 (16.9%) | 89 |
| | High BMI Male | Low | 26 (29.2%) | 89 |

The distribution of SMI and SMD by sex, BMI where appropriate, and patient type are shown in Figure 5.5, and Figure 5.6 respectively. The distributions shown are detailed in Table 5.4 for SMI and in Table 5.5 for SMD. This reveals a startling prevalence of sarcopenia amongst healthy (LDN) patients, with 21 of 53 (39.6%) of selected healthy kidney donors exhibiting CT-BCA sarcopenia. Patients of AAA type also exhibited CT-BCA signs of sarcopenia, with 29 of 52 (55.8%) exhibiting CT-BCA sarcopenia. Interestingly 42 of 89 (47.2%) UGIC patients exhibited CT-BCA sarcopenia, revealing that AAA patients had greater prevalence of sarcopenia than UGIC patients.

Testing did not reveal a relationship between SMI and more advanced disease stage in the UGIC group.

The relationship between SMI, SMD, and BMI/weight-loss stage was considered as described in Chapter 3. In this case, the BMI/weight-loss stage as described by Martin *et al* [19] was applied across the entire cohort, to each group, and by sub-groups to investigate SMI and SMD. It was initially possible to demonstrate differences in SMI and SMD across the whole cohort between patients in different BMI/weight-loss classes. Weight, weight-loss percent, and BMI also showed significant differences between classes however as these parameters formed part of the class calculation they were disregarded. Upon looking more closely into patient groups, however, the differences in SMI and SMD disappeared.

Table 5.5: Number and Percentage of Patients of each Type by CT Radio-Density

| Patient Type | Sex/BMI | Radio-Density by SMD | n(%) per Type | Total per Type |
|---|---|---|---|---|
| **Live donor** | | | | |
| | Low BMI Male | Normal | 7 (13.2%) | 53 |
| | Low BMI Male | Low Radio-Density | 2 (3.8%) | 53 |
| | High BMI Male | Normal | 12 (22.6%) | 53 |
| | High BMI Male | Low Radio-Density | 3 (5.7%) | 53 |
| | Low BMI Female | Normal | 8 (15.1%) | 53 |
| | Low BMI Female | Low Radio-Density | 2 (3.8%) | 53 |
| | High BMI Female | Normal | 16 (30.2%) | 53 |
| | High BMI Female | Low Radio-Density | 3 (5.7%) | 53 |
| **Vascular** | | | | |
| | Low BMI Male | Normal | 8 (15.4%) | 52 |
| | Low BMI Male | Low Radio-Density | 4 (7.7%) | 52 |
| | High BMI Male | Normal | 11 (21.2%) | 52 |
| | High BMI Male | Low Radio-Density | 21 (40.4%) | 52 |
| | Low BMI Female | Low Radio-Density | 4 (7.7%) | 52 |
| | High BMI Female | Normal | 3 (5.8%) | 52 |
| | High BMI Female | Low Radio-Density | 1 (1.9%) | 52 |
| **Cancer** | | | | |
| | Low BMI Male | Normal | 9 (10.1%) | 89 |
| | Low BMI Male | Low Radio-Density | 12 (13.5%) | 89 |
| | High BMI Male | Normal | 21 (23.6%) | 89 |
| | High BMI Male | Low Radio-Density | 20 (22.5%) | 89 |
| | Low BMI Female | Normal | 3 (3.4%) | 89 |
| | Low BMI Female | Low Radio-Density | 8 (9%) | 89 |
| | High BMI Female | Normal | 9 (10.1%) | 89 |
| | High BMI Female | Low Radio-Density | 7 (7.9%) | 89 |

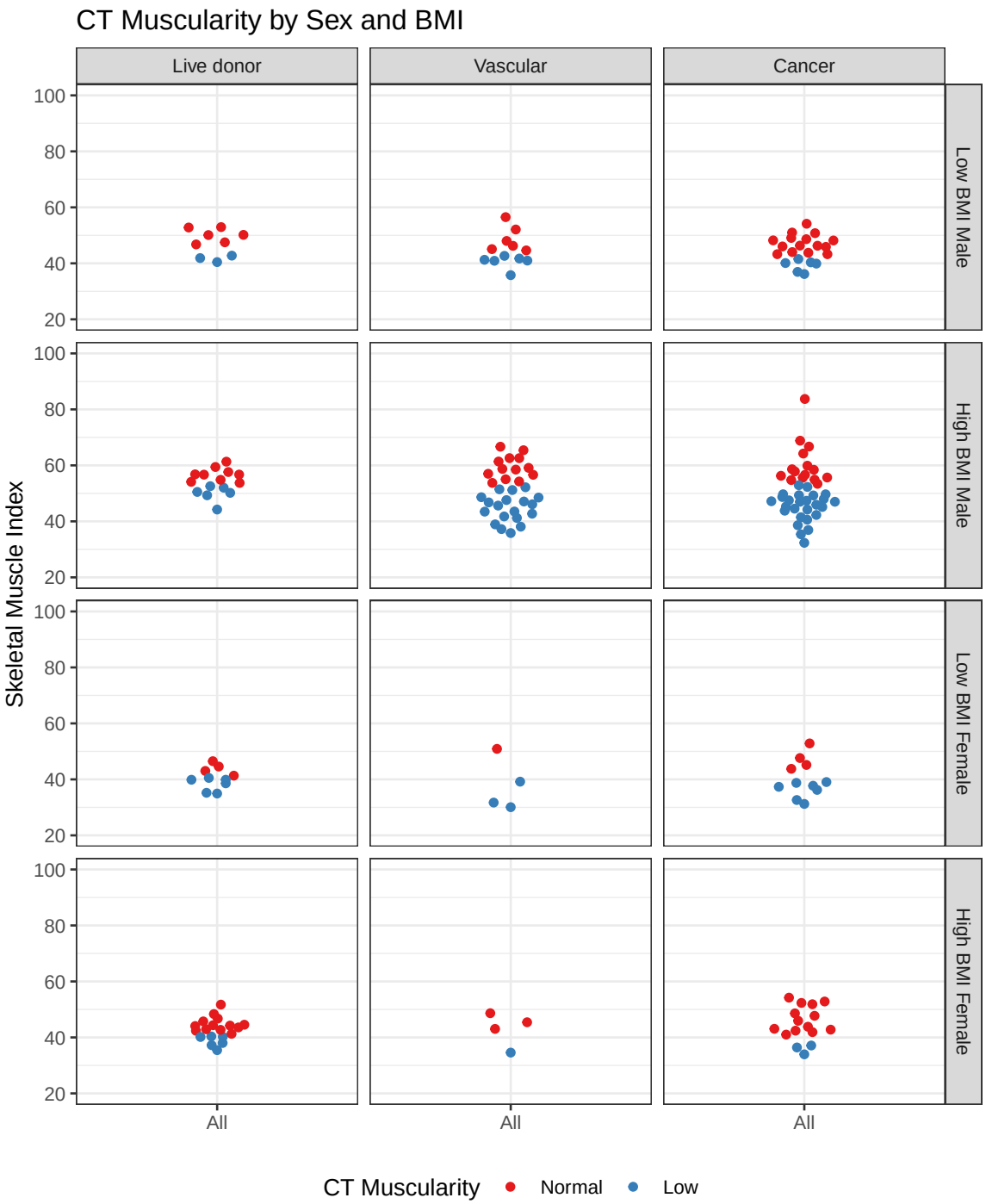



Figure 5.5: Patient SMI and Sarcopenia by Group, Sex, and BMI

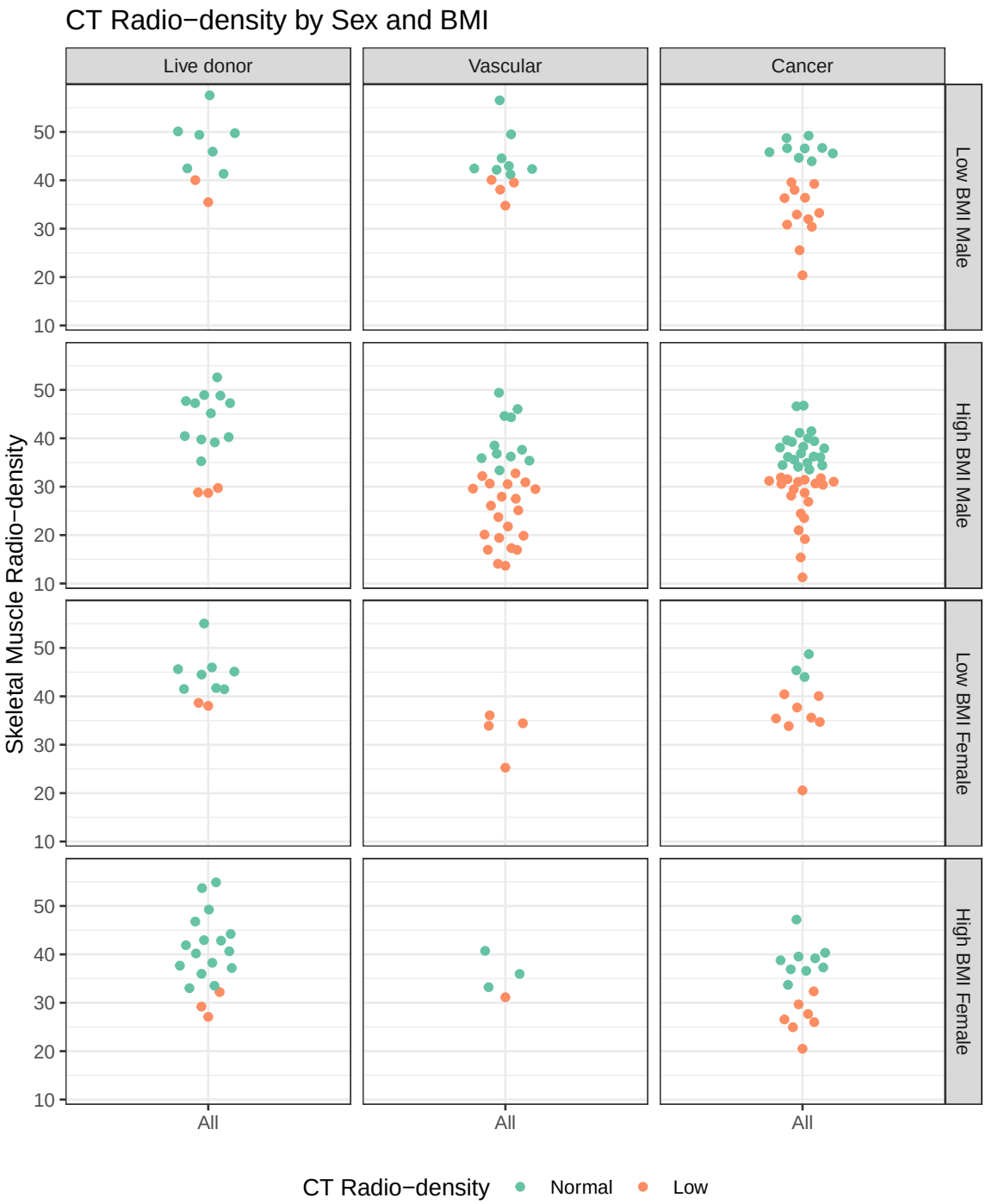



Figure 5.6: Patient SMD by Group, Sex, and BMI

### 5.3.3 Applying cancer cachexia criteria to non-cancer patients

The international consensus definition of cachexia includes both CT and weight-loss criteria [12], as discussed in greater depth in Chapter 1 with this classification being applied as discussed in Chapter 3. Whilst accepting that the definition applies to cancer patients, it seemed reasonable as a point of interest to ascertain how many of the other non-cancer patients may fall under the diagnostic criteria.

In performing the analysis, the LDN group were excluded, as although many of these patients reported weight loss, this was intentional and designed to either allow them entry to the LDN programme, or to speed their recovery after surgery.

The AAA cohort did not report intentional weight loss, but many did report that their weight had decreased and given that a visible proportion of these patients had low SMI by published criteria they were included in the analysis. This is shown in Figure 5.7.

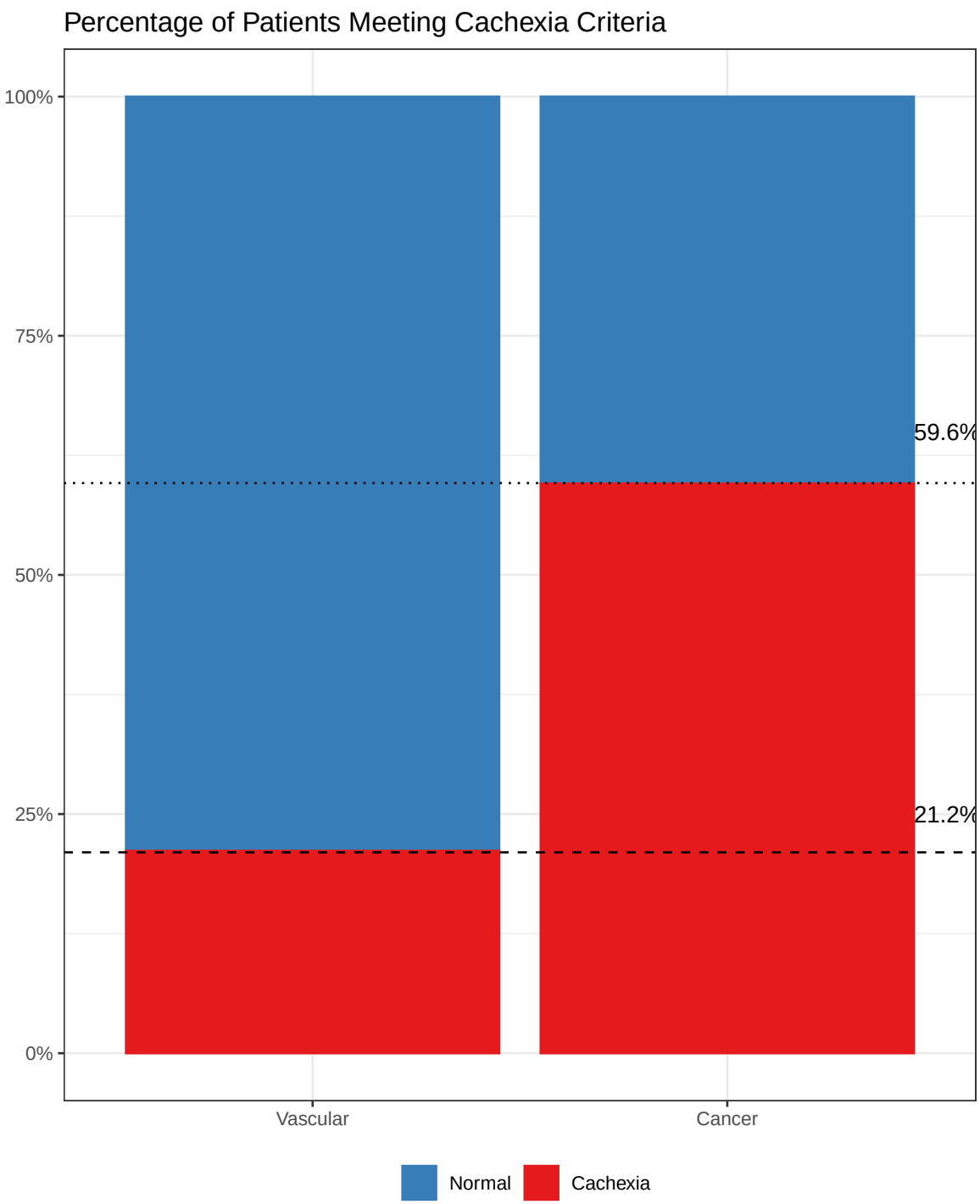



Figure 5.7: Percentage of Patients Meeting Cachexia Criteria

### 5.3.4 Multivariable Analysis of CT-BCA

As described in Chapter 3, a multivariable analysis was conducted to ascertain any significant relationships between CT-BCA and patient phenotype. Stepwise model construction to predict SMI identified group, BMI, sex, SMD, Eastern Clinical Oncology Group (ECOG) score, weight, weight loss (WL) category, weight loss percentage, and Edmonton frail scale (EFS) category as being of potential interest in relation to SMI. This model is shown in Table 5.6.

When considering the effect of each of these predictors for SMI, it is important to note that within the WL category predictor, the 2% WL + low muscularity (LM) category includes SMI as part of the definition, and so whilst there is a significance relating to this it should be considered in this context. Additionally, when considering BMI it has to be remembered that SMI is indexed for height in a similar fashion to BMI and so this should be noted when interpreting the model. This leaves sex and ECOG score 2 as the remaining predictors of SMI. As noted above, there is a difference in SMI by sex, with females having lower SMI. ECOG score 2 relates to reduced physical activity, which is an understandable potential predictor of SMI. Additionally, the Akaike information criterion suggests that this is not a well-fitting model.

Similarly, stepwise model construction was carried out to predict SMD. This identified age, sex, weight, SMI, and ECOG score as being of potential interest, and the model is shown in Table 5.7.

When considering the significance of each of the SMD potential predictors, these do appear slightly easier to explain. Age, female sex, weight, and SMI all appear to be statistically significant components of a multivariable SMD prediciton model. The Akaike information criterion assessing fitting of the model, however, reveals a poorly fitting model.

Table 5.6: Multivariable Analysis Predicting SMI

| Dependent: SMI | | unit | value | Coefficient (univariable) | Coefficient (multivariable) |
|---|---|---|---|---|---|
| Group | Live donor | Mean (sd) | 46.3 (6.7) | - | - |
| | Vascular | Mean (sd) | 47.7 (8.7) | 1.37 (-1.80 to 4.54, p=0.394) | -2.50 (-5.82 to 0.82, p=0.139) |
| | Cancer | Mean (sd) | 46.9 (8.6) | 0.61 (-2.06 to 3.28, p=0.653) | 0.93 (-2.00 to 3.85, p=0.532) |
| BMI | [15.0,48.0] | Mean (sd) | 46.9 (8.2) | 0.81 (0.63 to 0.99, p<0.001) | 1.34 (0.84 to 1.84, p<0.001) |
| Sex | Male | Mean (sd) | 49.2 (8.2) | - | - |
| | Female | Mean (sd) | 42.0 (5.6) | -7.14 (-9.28 to -5.01, p<0.001) | -9.77 (-13.06 to -6.49, p<0.001) |
| SMD | [11.3,59.5] | Mean (sd) | 46.9 (8.2) | 0.04 (-0.08 to 0.16, p=0.473) | 0.22 (0.08 to 0.37, p=0.003) |
| ECOG SCore | 0 | Mean (sd) | 46.8 (6.9) | - | - |
| | 1 | Mean (sd) | 48.1 (9.7) | 1.30 (-1.17 to 3.77, p=0.299) | 0.33 (-2.08 to 2.74, p=0.786) |
| | 2 | Mean (sd) | 36.2 (5.5) | -10.61 (-18.60 to -2.63, p=0.009) | -9.83 (-16.79 to -2.86, p=0.006) |
| | 3 | Mean (sd) | 44.9 (11.0) | -1.95 (-9.13 to 5.22, p=0.592) | -3.00 (-9.60 to 3.61, p=0.370) |
| Weight | [38.0,137.6] | Mean (sd) | 46.9 (8.2) | 0.22 (0.17 to 0.27, p<0.001) | -0.14 (-0.31 to 0.03, p=0.101) |
| WL Category | Weight Stable | Mean (sd) | 47.9 (8.6) | - | - |
| | 2% WL + LM | Mean (sd) | 40.8 (6.1) | -7.10 (-10.98 to -3.23, p<0.001) | -7.57 (-11.16 to -3.97, p<0.001) |
| | 5% WL | Mean (sd) | 46.8 (7.2) | -1.12 (-3.40 to 1.17, p=0.335) | -4.20 (-7.56 to -0.83, p=0.015) |
| Percentage Weight Loss | [-18.1,51.2] | Mean (sd) | 46.9 (8.2) | -0.03 (-0.16 to 0.10, p=0.603) | 0.14 (-0.02 to 0.29, p=0.079) |
| EFS Category | No Frailty | Mean (sd) | 46.8 (7.5) | - | - |
| | Pre-frail | Mean (sd) | 49.8 (13.9) | 3.01 (-2.08 to 8.11, p=0.244) | 2.47 (-1.34 to 6.28, p=0.202) |
| NA | NA | NA | NA | 14.55 (-1.67 to 30.78, p=0.078) | 18.68 (5.21 to 32.16, p=0.007) |

Number in dataframe = 226, Number in model = 127, Missing = 99, Log-likelihood = -386.96, AIC = 805.9, R-squared = 0.61, Adjusted R-squared = 0.57

Table 5.7: Multivariable Analysis Predicting SMD

| Dependent: SMD | | unit | value | Coefficient (univariable) | Coefficient (multivariable) |
|---|---|---|---|---|---|
| Age | [25.0,90.0] | Mean (sd) | 37.8 (9.0) | -0.36 (-0.44 to -0.27, p<0.001) | -0.33 (-0.40 to -0.25, p<0.001) |
| Sex | Male | Mean (sd) | 37.4 (9.5) | - | - |
| | Female | Mean (sd) | 38.8 (7.7) | 1.42 (-1.13 to 3.98, p=0.273) | -2.28 (-4.42 to -0.14, p=0.037) |
| Weight | [38.0,137.6] | Mean (sd) | 37.8 (9.0) | -0.21 (-0.26 to -0.15, p<0.001) | -0.31 (-0.37 to -0.26, p<0.001) |
| SMI | [30.1,83.7] | Mean (sd) | 37.8 (9.0) | 0.05 (-0.09 to 0.20, p=0.473) | 0.23 (0.11 to 0.36, p<0.001) |
| ECOG SCore | 0 | Mean (sd) | 39.5 (8.6) | - | - |
| | 1 | Mean (sd) | 34.3 (8.9) | -5.25 (-7.94 to -2.57, p<0.001) | -2.81 (-4.73 to -0.89, p=0.004) |
| | 2 | Mean (sd) | 27.9 (1.2) | -11.59 (-20.27 to -2.91, p=0.009) | -4.49 (-10.75 to 1.77, p=0.159) |
| | 3 | Mean (sd) | 32.5 (9.9) | -7.00 (-14.79 to 0.79, p=0.078) | -7.12 (-12.56 to -1.68, p=0.011) |
| Number in dataframe = 226, Number in model = 194, Missing = 32, Log-likelihood = -619.9, AIC = 1257.8, R-squared = 0.57, Adjusted R-squared = 0.56 | | | | | |

Table 5.8: Calculations Used to Derive New SMI Cut-Point

| Sex | BMI | Min SMI | Mean SMI | Max SMI | SD | Mean - (2SD) |
|---|---|---|---|---|---|---|
| **Male** | | | | | | |
| | Normal | 40.4 | 47.3 | 52.9 | 4.7 | 37.9 |
| | High | 44.2 | 54.0 | 61.3 | 4.4 | 45.2 |
| **Female** | | | | | | |
| | Normal | 34.9 | 40.4 | 46.5 | 3.7 | 33.1 |
| | High | 35.5 | 42.8 | 51.7 | 3.9 | 35.0 |

### 5.3.5 Deriving CT-BCA cut-points from current cohort

Having considered the application of previously published cut-points to the current cohort, attention was turned to applying cut-points derived from the healthy controls recruited to the present study. It is known that the number of patients used to define normality in laboratory tests are orders of magnitude greater than the numbers present in this study. Indeed, when considering for example blood tests the reference range may be derived from many tens of thousands of patient tests. Whilst clearly there are far fewer patients in the present study, nevertheless some inferences can be made. The original work defining sarcopenia [7] used patients from a previous study [94] which included 68 females and 64 males. This work defined sarcopenia in terms of DEXA scanning results, and the translation to CT, performed by Mourtzakis *et al*[17] used 31 patients with concurrent CT and DEXA to provide CT-BCA cut-points (Male $<55.4 cm^2/m^2$, Female $<38.9 cm^2/m^2$). The inclusion of BMI as part of CT-BCA sarcopenia assessment was performed by Martin *et al*[18] using 1473 patients and analysing patient outcome to stratify into those with good or poor outcome (High BMI male $<53 cm^2/m^2$, Normal BMI Male $<43 cm^2/m^2$, High BMI Female $<41 cm^2/m^2$, Normal BMI Female $<41 cm^2/m^2$). Both the Mourtzakis *et al* and the Martin *et al* studies used cancer patients to define their equations and cut-points. As such, there has not been a series of healthy patients with CT-BCA performed to define cut-points using the methods described by Baumgartner *et al*[7] until now. The de-novo cut-points were derived as described in Chapter 3, subtracting two standard deviations (SD) from the mean, revealing calculations as detailed in Table 5.8. It is to be noted that for some patient subgroups - specifically females - the *"Mean - (2SD)"* value is actually below the minimum range of the data.

The results of this reveal the cut-points below, and as shown in Table 5.9 which includes previous cut-points for comparison.

- 37.9 $cm^2/m^2$ for normal BMI males,
- 45.2 $cm^2/^{m2}$ for high BMI males,

Table 5.9: Previously Published and Newly Calculated SMI Cut-Point Definitions

| Cut-Point Source | Year | Male | High BMI Male | Female | High BMI Female |
|---|---|---|---|---|---|
| Mourtzakis | 2008 | 55.4 | | 38.9 | |
| Consensus Definition | 2011 | 55 | | 39 | |
| Martin | 2013 | 43 | 53 | 41 | 41 |
| New | 2019 | 37.9 | 45.2 | 34.14 | 35.0 |

- 33.1 $cm^2/m^2$ for normal BMI females, and
- 35.0 $cm^2/m^2$ for high BMI females

The breakdown of patients into each group is shown in Table 5.10 in numerical form, and in Figure 5.8 in a graphical form.

Table 5.10: Patient Distribution by New Cut-Point

| Patient Type | Sex | New Cut-point SMI | n | Total (Percentage) Per Sex | Total Patients Per Type |
|---|---|---|---|---|---|
| **Live donor** | | | | | |
| | Male | Normal | 23 | 24 (95.8%) | 53 |
| | Male | Low | 1 | 24 (4.2%) | 53 |
| | Female | Normal | 29 | 29 (100%) | 53 |
| **Vascular** | | | | | |
| | Male | Normal | 34 | 44 (77.3%) | 52 |
| | Male | Low | 10 | 44 (22.7%) | 52 |
| | Female | Normal | 5 | 8 (62.5%) | 52 |
| | Female | Low | 3 | 8 (37.5%) | 52 |
| **Cancer** | | | | | |
| | Male | Normal | 49 | 62 (79%) | 89 |
| | Male | Low | 13 | 62 (21%) | 89 |
| | Female | Normal | 24 | 27 (88.9%) | 89 |
| | Female | Low | 3 | 27 (11.1%) | 89 |

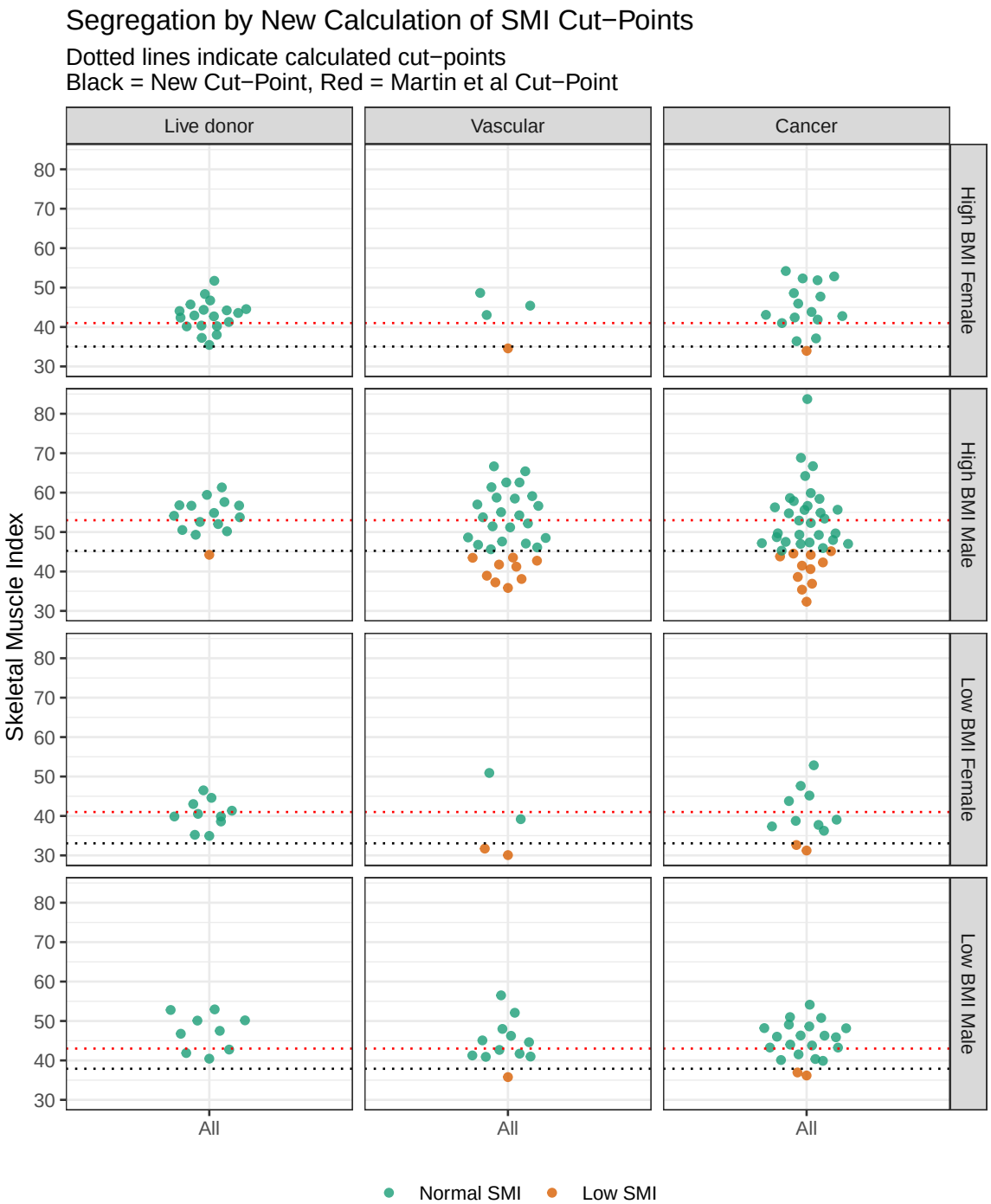



Figure 5.8: Distribution of Patients by Type and SMI by New Cut-point

## 5.4 Discussion of CT use in Defining Cachexia and Sarcopenia

The figures presented in Table 5.1 show some differences between CT muscularity groups which are to be expected, and some which are less obvious. A statistically significant increase in age in the low muscularity group is in keeping with the development of sarcopenia as age increases [7]. The histogram in Figure 5.1 clearly shows that within each sex, there are marked and obvious differences in SMI distribution, even though these do not reach statistical significance. If the LDN population were to be considered as *"normal"*, then it can clearly be seen that male UGIC patients have a lower SMI distribution by comparison. Similarly, male AAA patients have a lower mean SMI than LDN patients, but also a lower mean SMI than the UGIC group. This would suggest that the presence of either upper GI cancer, or aortic vascular disease, has a notable reduction effect on the indexed muscularity of males. Whilst the mechanism of this reduction in both groups is not fully understood, it could be inferred that there is a common pathway resulting in loss of muscle mass independent of a particular disease process. This is clearly important when considering the ongoing efforts to find both a mechanism for the development of cachexia, and following on from this a target for intervention. It may be that by comparing underlying biochemistry between both cancer and non-cancer patients, a potentially unifying pathway may be derived.

When considering the female patients, the differences are less clear. The LDN group do appear to have a greater density of patients with similar SMI, whilst the UGIC and to a lesser degree AAA patients have an increased variability of muscularity. Whilst this may be a reflection of the relative numbers of female patients within each group, nevertheless it could be expected that although histographic plot density peaks may be smaller they should occur at around the same point in the absence of a differentiating factor - in this case aortic vascular disease. Again, the clustering of LDN SMI could be considered to be *"normal"*, meaning that the widening spread of SMI in the cancer and non-cancer disease cohorts may be an effect of the underlying disease process. Interesting differences appear when patient SMD is segregated not only by sex but also by patient type. As can be seen in Figure 5.3, both Male and Female patients show a marked decrease in SMD when moving from *"healthy"* through *"non-cancer, vascular"* to *"cancer"* patients (despite this not being a continuum). There is a clear reduction in CT SMD between the LDN and the AAA group, and more markedly between the LDN and the UGIC group. This is echoed in both males and females and suggests that for SMD there is an effect of disease on SMD. Noting the distribution of the density curves, it can be seen that both disease states studied confer a reduction in SMD. This is confirmed by the statistically significant differences in SMD between groups as shown above.

In considering the proposal that cachexia is an inexorable disease-related process resulting in muscle loss [12], and with non-cancer research groups maintaining similar proposals [92], it could be expected that both the LDN and AAA groups in this cohort would naturally have a higher SMI, and fewer sarcopenic patients, than the cancer group. As can be seen in Figure 5.5, and in Table 5.1, this is not the case.

Of note, cachexia as a result of aortic vascular disease has not been described previously.

In trying to ascertain the reasons for these demonstrated differences in CT-BCA, both methodological and population-specific factors must be taken into account.

In terms of methodology, the production of cut-points to predict prognosis from SMI make an assumption that low SMI is a driving factor for the deterioration of patients with cancer. The assumption reasons that low SMI means more advanced or aggressive disease and that is is the disease driving the low SMI and thus the poor prognosis. If this were to be considered in the reverse, however, it may be that the findings of the current study may make more sense. If as seen in the various histogram plots noted above there is a relatively normal distribution of SMI in patients irrespective of disease state, then perhaps the SMI merely represents the wide variety of the human condition. If so, it may then follow that in the preoperative phase of disease such a distribution would not be unexpected, as these patients have yet to progress towards relentless skeletal muscle loss. Were this to be the case, the observed similarities between the cancer and non-cancer groups would not be unexpected. Accordingly, any cut-points derived from pre-operative cancer populations would need to account for the preservation of functional state in these specific groups. Additionally, it may be that the patients in whom prognosis was poor represented those who were already on a deteriorating pathway and had already lost significant muscle mass and volume prior to analysis. If so, the tumour biology in these patients may be somewhat more aggressive resulting in faster muscle loss and death, meaning that the low SMI seen to give poor prognosis is in fact a reflection of the underlying disease rather than a contributor to it.

Although some conclusions may be drawn from the present study, it is important to recognise that the data colected represent a *"snapshot"* of the patient's journey. Thus it is impossible to ascertain with precision prior muscularity, function m and symptomatology. Equally importantly, if not more so, is the weakness inherent in the absence of longitudinal measures of patient function, weight loss or gain, symptomatology over time, and survival. Accordingly, whilst there are worthwhile observations to be made, it should be remembered that, in the words of Aristotle [171]:

*"one swallow does not make a summer, nor does one day; and so too one day, or a short time, does not make a man blessed and happy"*

In terms of population-specific factors, it is known that worldwide different populations have differing

musculature and that the application of a cut-point defined in one population to another may be inappropriate. With this in mind, it may be that rather than a single unifying cut-point for wide populations (e.g. Caucasian, African, Asian etc..) being applicable, a more local solution is required for each country or even county. With regards to the Martin *et al* cut-points, these were derived from a Canadian registry as described by Prado *et al* [65]. This dataset describes patients attending a particular hospital in northern Alberta, in which the Caucasian population is around 70% [123]. It could be expected that in this context the muscularity differences related to race or ethnic origin between the published cut-points and the current cohort would be limited, however in addition the increasingly sedentary lifestyle and exercise choices of the Scottish population need to be taken into account. Clearly a more active population will in general retain their musculature in the absence of disease more than a sedentary population and this may be a contributing factor to the high levels of sarcopenia seen in "healthy" kidney donors. Nevertheless, the difference in cut-points generated in the current healthy control cohort and those derived from cancer patients is marked.

When considering SMD, the problem is rather more subtle. Knowing what a normal SMD is, and what it represents is not certain especially in the field of cancer cachexia and sarcopenia. Interpreting deviations from a perceived normal, therefore, is fraught with confounders. Firstly, it is unclear from where the normal came, and in what population, and of what age. Although apparently specified, the demographics - upon which so much will change and depend - are open to interpretation and depend upon the reader dissecting the Canadian census to ascertain how many of the patients studied could potentially belong to a specific demographic, geographic, and racial group. Further, and as discussed elsewhere, the impact of systemic inflammation (SI) on the amount of tissue oedema present is unquantified in general, and is remarked in Martin *et al* specifically [18]. An increase in tissue oedema will increase tissue water, and result in decreased radio-attenuation. This remains to be addressed in the cachexia and sarcopenia literature, and is discussed in this thesis in Chapter 9. Additionally, the relationship between SMD and muscle protein content is far from clear or linear, as discussed in a following chapter, and thus other components of skeletal muscle and its surroundings within the tomographic field must be considered. As suggested by Rollins *et al* [68], the replacement of muscle with fat may be a significant contributing factor in cachexia, and will undoubtedly have an effect on muscle radio-density. As yet, it is unclear how to best differentiate muscle and intramuscular fat on CT, particularly given the afore-mentioned confounding factors.

# Chapter 6

# Relationship Between CT Variables, Functional Status, and Quality Of Life

As described in the earlier introduction, there is an increasing interest in patient-reported outcome measures (PROMs). PROMs evince patient quality of life (QoL) and as such can reflect the benefit or otherwise of disease-related interventions in a patient-centred way unrelated to objective disease control measures such as tumour clearance. Additionally, it is possible not only to quantify the QoL of patient groups but also to ascertain the sensitivity of the questionnaires used in detecting QoL changes in a population whose health is sufficiently preserved to undergo surgical intervention.

## 6.1 Hypothesis and Research Questions

### 6.1.1 Hypothesis

CT-BCA variables relate well to patient-reported QoL and objective functional measures and can differentiate between groups

### 6.1.2 Research Questions

- How well-preserved or otherwise is QoL in a preoperative patient group?
- Can CT-BCA cut-points divide QoL into patient groups with higher or lower QoL scores?
- Can CT-BCA cut-points divide objective functional measurements into patient groups meeting or failing to meet published function targets?

## 6.2 Methods of Assessing QoL and CT variables

Between December 2015 and August 2017, 197 surgical patients were recruited to an observational study investigating the relationship between body composition, and QoL. Patients were from one of 3 distinct groups: Upper GI cancer (UGIC) including those with oesophageal, gastric, and pancreatic cancer; vascular aortic patients (AAA); and live kidney donors (LDN). Each patient was known to have had a pre-operative CT scan which was analysed at L3 level using semi-automated Slice-O-Matic software v4.2 (Tomovision Montreal-Canada), defining area measurements of skeletal muscle, subcutaneous fat, and visceral fat. These

area measurements were indexed for patient height as described in Chapter 3, analysed and plotted using R [168]. As CT scan phase is known to have an impact upon the radio-density of skeletal muscle as found by van Vugt *et al* [61], the equations developed by Rollins *et al* [60] from a cohort of pancreatic cancer patients undergoing triple-phase CT scans were applied. Specifically, the equation to convert arterial phase SMD to portal venous phase SMD was applied where appropriate:

$$SMHUpv=(0.979*SMHUa)+3.213$$

Quality of life metrics were assessed using the *"EORTC QLQ-C30+OG25"* and *"EQ5D-3L"* questionnaires. With regards to the *QLQ-C30+OG25* scoring, in line with the instruction manual the scores are divided into symptomatic and functional domains. When scoring symptomatic domains, a lower score indicates fewer symptoms or a better score within that symptomatic domain. When scoring functional domains, a higher score indicates fewer functional impairments or a better score within that functional domain. Accordingly, a patient with preserved quality of life would be expected to exhibit a low symptomatic domain score and a high functional domain score. To increase ease of interpretation graphically, scales were assigned such that points nearer the circumference indicated better performance, and QoL reduced towards the centre point of each chart. Patient functioning was assessed using the Edmonton Frail Scale (EFS), the reported EFS (REFS), the patient-generated subjective global assessment (PG-SGA), and the timed up-and-go test (TUG).

## 6.3 Results of CT and QoL Analysis

Functional assessment data are presented in Table 6.1, and QoL data in Table 6.2 for symptomatic domains and in Table 6.3 for functional domains.

Table 6.1: Demographic distribution of functional assessment

| Dependent: Patient Group | | Live donor | Vascular | Cancer | p |
|---|---|---|---|---|---|
| Sex | Male | 24 (45.3) | 44 (84.6) | 62 (69.7) | <0.001 |
| | Female | 29 (54.7) | 8 (15.4) | 27 (30.3) | |
| Age (Years | Mean (SD) | 51.7 (12.2) | 66.5 (10.4) | 67.7 (9.3) | <0.001 |
| BMI | Mean (SD) | 25.6 (3.1) | 27.2 (5.1) | 26.9 (5.8) | 0.178 |
| Height (cm) | Mean (SD) | 168.9 (10.0) | 173.9 (9.8) | 171.4 (9.9) | 0.040 |
| Weight (Kg) | Mean (SD) | 73.2 (13.0) | 83.6 (21.7) | 79.1 (18.5) | 0.015 |
| Percentage Weight Loss | Mean (SD) | 3.7 (10.7) | 1.4 (6.1) | 6.6 (7.6) | 0.001 |
| Gait Speed (m/s) | Mean (SD) | 1.6 (0.3) | 1.3 (0.4) | 1.4 (0.3) | <0.001 |
| Timed Up-And-Go (s) | Mean (SD) | 7.9 (1.2) | 10.3 (3.4) | 9.0 (2.3) | <0.001 |
| SMI | Mean (SD) | 46.3 (6.7) | 47.7 (8.7) | 47.0 (8.5) | 0.688 |
| SMD (HU) | Mean (SD) | 42.1 (7.2) | 33.3 (9.8) | 34.9 (7.9) | <0.001 |
| ASA | 1 | 42 (79.2) | | | <0.001 |
| | 2 | 11 (20.8) | 3 (6.1) | 50 (58.8) | |
| | 3 | | 44 (89.8) | 35 (41.2) | |
| | 4 | | 2 (4.1) | | |
| ECOG | 0 | 52 (98.1) | 23 (44.2) | 50 (56.2) | <0.001 |
| | 1 | 1 (1.9) | 23 (44.2) | 36 (40.4) | |
| | 2 | | 2 (3.8) | 2 (2.2) | |
| | 3 | | 4 (7.7) | 1 (1.1) | |

*Note:*

NaN indicates missing values, NA indicates the test was unable to be run. Tests used include Wilcoxon-Kruskal-Wallis test for continuous variables, Pearson chi-square test for categorical variables, and the likelihood ratio chi-square test from the proportional odds model for ordinal variables.

Table 6.2: Demographic Distribution of All Quality of Life Symptomatology Data

| Dependent: cancercontrol | | Live donor | Vascular | Cancer | p |
|---|---|---|---|---|---|
| Quality of life overall | Mean (SD) | 6.0 (10.7) | 30.1 (22.2) | 29.0 (22.8) | <0.001 |
| Fatigue | Mean (SD) | 3.2 (6.9) | 25.5 (28.8) | 20.9 (22.2) | <0.001 |
| Nausea and Vomiting | Mean (SD) | 0.0 (0.0) | 4.5 (15.5) | 11.9 (19.6) | <0.001 |
| Pain | Mean (SD) | 3.0 (9.9) | 24.0 (33.1) | 12.8 (21.0) | <0.001 |
| Dyspnoea | Mean (SD) | 1.3 (6.6) | 17.0 (29.3) | 8.2 (17.9) | 0.001 |
| Insomnia | Mean (SD) | 11.3 (19.8) | 30.1 (35.7) | 25.9 (30.7) | 0.004 |
| Appetite Loss | Mean (SD) | 0.0 (0.0) | 13.1 (25.9) | 24.3 (34.6) | <0.001 |
| Constipation | Mean (SD) | 1.3 (6.6) | 7.1 (15.2) | 14.0 (23.5) | 0.001 |
| Diarrhoea | Mean (SD) | 2.0 (8.0) | 3.2 (9.9) | 11.9 (21.9) | 0.001 |
| Financial Difficulties | Mean (SD) | 0.0 (0.0) | 16.3 (32.9) | 8.6 (23.4) | 0.003 |
| Dysphagia | Mean (SD) | 0.0 (0.0) | 2.5 (8.9) | 20.0 (27.9) | <0.001 |
| Eating | Mean (SD) | 0.0 (0.0) | 10.6 (23.0) | 28.2 (27.6) | <0.001 |
| Reflux | Mean (SD) | 2.3 (5.8) | 8.7 (14.9) | 14.2 (22.2) | 0.001 |
| Odynophagia | Mean (SD) | 0.0 (0.0) | 4.2 (13.6) | 18.8 (28.6) | <0.001 |
| Pain and discomfort | Mean (SD) | 2.0 (7.3) | 14.1 (25.4) | 18.3 (25.8) | <0.001 |
| Anxiety | Mean (SD) | 4.0 (9.9) | 43.3 (28.8) | 55.4 (27.1) | <0.001 |
| Eating with others | Mean (SD) | 0.0 (0.0) | 1.9 (13.9) | 13.3 (30.3) | 0.001 |
| Dry Mouth | Mean (SD) | 2.0 (8.0) | 16.0 (26.8) | 26.7 (32.0) | <0.001 |
| Trouble with taste | Mean (SD) | 0.0 (0.0) | 5.2 (18.1) | 15.8 (26.5) | <0.001 |
| Trouble swallowing saliva | Mean (SD) | 1.3 (6.6) | 0.7 (4.7) | 11.5 (27.0) | 0.001 |
| Choked when Swallowing | Mean (SD) | 0.0 (0.0) | 3.2 (15.1) | 8.2 (20.8) | 0.014 |
| Trouble with coughing | Mean (SD) | 7.3 (13.9) | 19.9 (21.1) | 28.7 (27.4) | <0.001 |
| Trouble Talking | Mean (SD) | 0.0 (0.0) | 1.3 (6.5) | 4.2 (12.3) | 0.025 |
| Weight Loss | Mean (SD) | 0.0 (0.0) | 4.5 (17.5) | 21.0 (32.3) | <0.001 |
| Hair Loss | Mean (SD) | 0.0 (0.0) | 0.0 (0.0) | 7.5 (14.2) | 0.005 |

Table 6.2: Demographic Distribution of All Quality of Life Symptomatology Data *(continued)*

| Dependent: cancercontrol | Live donor | Vascular | Cancer | p |
|---|---|---|---|---|

*Note:*

NaN indicates missing values, NA indicates the test was unable to be run. Tests used include Wilcoxon-Kruskal-Wallis test for continuous variables, Pearson chi-square test for categorical variables, and the likelihood ratio chi-square test from the proportional odds model for ordinal variables.

Table 6.3: Demographic Distribution of All Quality of Life Functional Data

| Dependent: Patient Group | | Live donor | Vascular | Cancer | p |
|---|---|---|---|---|---|
| Physical Functioning | Mean (SD) | 98.5 (4.7) | 81.5 (20.9) | 92.5 (11.3) | <0.001 |
| Role Functioning | Mean (SD) | 100.0 (0.0) | 71.8 (35.8) | 88.1 (22.4) | <0.001 |
| Emotional Functioning | Mean (SD) | 93.0 (10.0) | 81.3 (23.8) | 78.9 (22.8) | 0.001 |
| Cognitive Functioning | Mean (SD) | 97.7 (5.8) | 86.2 (25.7) | 87.2 (18.7) | 0.003 |
| Social Functioning | Mean (SD) | 100.0 (0.0) | 78.8 (27.6) | 84.0 (25.7) | <0.001 |
| Body Image | Mean (SD) | 100.0 (0.0) | 96.2 (15.7) | 86.7 (26.8) | <0.001 |

*Note:*

NaN indicates missing values, NA indicates the test was unable to be run. Tests used include Wilcoxon-Kruskal-Wallis test for continuous variables, Pearson chi-square test for categorical variables, and the likelihood ratio chi-square test from the proportional odds model for ordinal variables.

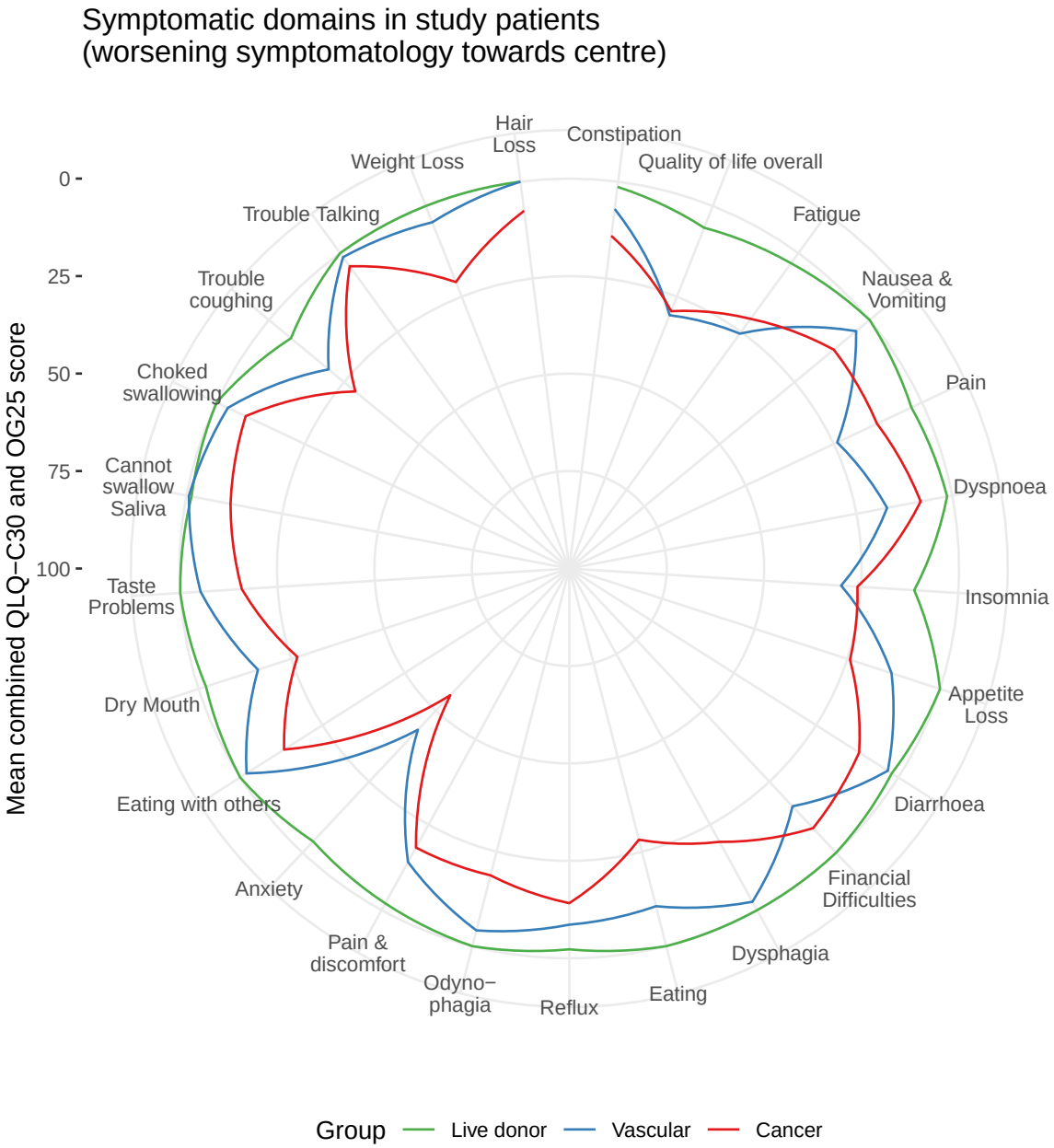



Figure 6.1: Quality of life Symptom Domains by Patient Type

### 6.3.1 Timing of Questionnaire Administration

As discussed in Chapter 3, it was not possible to be certain exactly which patients had completed QoL questionnaires before, and which after chemotherapeutic interventions in the UGIC group. To further

investigate this as a possible confounding factor, the time between consent and operation was examined. This showed a split in the UGIC group at around 50-days, suggesting that those with >50-days to operation underwent chemotherapy. An analysis of these subgroups revealed that symptomatic domains reached significance between over/under 50-days in "hair loss" only. Differences in functional domains reached significance in "role functioning" only. Time from consent to operation was mean 35.85 days, median 24 days, range 0-143 days. From this, it seems reasonable to extrapolate that those with earlier operations do not have substantially better or worse QoL than those with later operations - other than as noted above, and which could be in keeping with chemotherapeutic side effects. Of note, no other domains were affected.

### 6.3.2 Symptomatic QoL Domains

These data can also be demonstrated in plot form, as shown in Figure 6.1 and reveal that the majority of patients have preserved symptomatic domain scores. Initial interrogation of the patient groups revealed overall differences in symptomatic domain scores, as shown in Table 6.4. A subsequent comparison between patient groups, shown in Tables 6.5, 6.6, and 6.7 reveals that there were statistically significant differences between all 3 patient groups for certain domains. These differences also reached clinical significance for some of the symptomatic domains (defined by a difference in scores of >10 points) as discussed in Chapter 3.

As could be expected, the LDN group have the most preserved symptomatic domain responses compared to both the UGIC and the AAA groups. In fact, and as seen in Figure 6.1, the responses returned by the LDN group indicated an overall paucity of symptoms. This fits with the group being a selected healthy population, and provides a useful baseline against which to measure a more unwell group.

When looking at the comparison between symptoms reported by the UGIC and the AAA groups (in Table 6.7) it can be seen that in all domains reaching dual significance criteria apart from pain, the UGIC patients scored greater symptomatology. Amongst the explanations for this could be that due to the operability of the cancer group, their disease is at a relatively early stage and has not yet spread to adjacent structures or bone and thus was not causing significant pain.

When comparing symptomatic domains within groups split by whether the patients are above or below the Martin *et al* criteria for SMI and for SMD, the number of domains reaching both significance levels dropped considerably. In the UGIC group, *Financial Difficulties* alone were highlighted, and were experienced more in the *normal* SMI group than in the *low* SMI group. The pathophysiology behind this is unclear, and whilst it is possible to speculate that those with *normal* or preserved SMI may be working in manual jobs (perhaps

self-employed) and that this underlies the result, the data gathered do not allow hypotheses such as these to be tested. In the AAA group, *Appetite Loss* alone was experienced more in those with *low* SMD than *normal* SMD. Again, the pathophysiology behind this is unclear and hypothetical explanations for this difference were not readily available. No other domains in any group reached both significance levels for either SMI or SMD.

Table 6.4: ANOVA Comparison of Symptomatology Scores Across All Patient Groups

| Dependent: Patient Group | | Live donor | Vascular | Cancer | p |
|---|---|---|---|---|---|
| Quality of Life Overall | Mean (SD) | 6.0 (10.7) | 30.1 (22.2) | 29.0 (22.8) | <0.001 |
| Fatigue | Mean (SD) | 3.2 (6.9) | 25.5 (28.8) | 20.9 (22.2) | <0.001 |
| Nausea and Vomiting | Mean (SD) | 0.0 (0.0) | 4.5 (15.5) | 11.9 (19.6) | <0.001 |
| Pain | Mean (SD) | 3.0 (9.9) | 24.0 (33.1) | 12.8 (21.0) | <0.001 |
| Dyspnoea | Mean (SD) | 1.3 (6.6) | 17.0 (29.3) | 8.2 (17.9) | 0.001 |
| Insomnia | Mean (SD) | 11.3 (19.8) | 30.1 (35.7) | 25.9 (30.7) | 0.004 |
| Appetite Loss | Mean (SD) | 0.0 (0.0) | 13.1 (25.9) | 24.3 (34.6) | <0.001 |
| Consipation | Mean (SD) | 1.3 (6.6) | 7.1 (15.2) | 14.0 (23.5) | 0.001 |
| Diarrhoea | Mean (SD) | 2.0 (8.0) | 3.2 (9.9) | 11.9 (21.9) | 0.001 |
| Financial Difficulties | Mean (SD) | 0.0 (0.0) | 16.3 (32.9) | 8.6 (23.4) | 0.003 |
| Dysphagia | Mean (SD) | 0.0 (0.0) | 2.5 (8.9) | 20.0 (27.9) | <0.001 |
| Eating | Mean (SD) | 0.0 (0.0) | 10.6 (23.0) | 28.2 (27.6) | <0.001 |
| Reflux | Mean (SD) | 2.3 (5.8) | 8.7 (14.9) | 14.2 (22.2) | 0.001 |
| Odynophagia | Mean (SD) | 0.0 (0.0) | 4.2 (13.6) | 18.8 (28.6) | <0.001 |
| Pain and discomfort | Mean (SD) | 2.0 (7.3) | 14.1 (25.4) | 18.3 (25.8) | <0.001 |
| Anxiety | Mean (SD) | 4.0 (9.9) | 43.3 (28.8) | 55.4 (27.1) | <0.001 |
| Eating with others | Mean (SD) | 0.0 (0.0) | 1.9 (13.9) | 13.3 (30.3) | 0.001 |
| Dry Mouth | Mean (SD) | 2.0 (8.0) | 16.0 (26.8) | 26.7 (32.0) | <0.001 |
| Traouble with taste | Mean (SD) | 0.0 (0.0) | 5.2 (18.1) | 15.8 (26.5) | <0.001 |
| Trouble swallowing saliva | Mean (SD) | 1.3 (6.6) | 0.7 (4.7) | 11.5 (27.0) | 0.001 |
| Choked when Swallowing | Mean (SD) | 0.0 (0.0) | 3.2 (15.1) | 8.2 (20.8) | 0.014 |
| Trouble with coughing | Mean (SD) | 7.3 (13.9) | 19.9 (21.1) | 28.7 (27.4) | <0.001 |

Table 6.4: ANOVA Comparison of Symptomatology Scores Across All Patient Groups *(continued)*

| Dependent: Patient Group | | Live donor | Vascular | Cancer | p |
|---|---|---|---|---|---|
| Trouble Talking | Mean (SD) | 0.0 (0.0) | 1.3 (6.5) | 4.2 (12.3) | 0.025 |
| Weight Loss | Mean (SD) | 0.0 (0.0) | 4.5 (17.5) | 21.0 (32.3) | <0.001 |
| Hair Loss | Mean (SD) | 0.0 (0.0) | 0.0 (0.0) | 7.5 (14.2) | 0.005 |

Table 6.5: Welch 2-sided t-test of Mean Quality of Life Symptomatology Results Between Cancer and Live Donor Patients Reaching both Clinical and Statistical Significance

| Domain | Difference | Cancer | Live donor | Low CI | High CI | p-value |
|---|---|---|---|---|---|---|
| Quality of life overall | -23.012 | 6.000 | 29.012 | -28.845 | -17.180 | <0.001 |
| Fatigue | -17.628 | 3.222 | 20.850 | -22.888 | -12.369 | <0.001 |
| Nausea and Vomiting | -11.934 | 0.000 | 11.934 | -16.265 | -7.604 | <0.001 |
| Insomnia | -14.593 | 11.333 | 25.926 | -23.323 | -5.862 | 0.001 |
| Appetite Loss | -24.280 | 0.000 | 24.280 | -31.923 | -16.637 | <0.001 |
| Constipation | -12.658 | 1.333 | 13.992 | -18.158 | -7.159 | <0.001 |
| Dysphagia | -20.000 | 0.000 | 20.000 | -26.201 | -13.799 | <0.001 |
| Eating | -28.194 | 0.000 | 28.194 | -34.332 | -22.057 | <0.001 |
| Reflux | -11.833 | 2.333 | 14.167 | -17.027 | -6.640 | <0.001 |
| Odynophagia | -18.750 | 0.000 | 18.750 | -25.116 | -12.384 | <0.001 |
| Pain and discomfort | -16.333 | 2.000 | 18.333 | -22.407 | -10.259 | <0.001 |
| Anxiety | -51.417 | 4.000 | 55.417 | -58.036 | -44.798 | <0.001 |
| Eating with others | -13.333 | 0.000 | 13.333 | -20.072 | -6.594 | <0.001 |
| Dry Mouth | -24.667 | 2.000 | 26.667 | -32.116 | -17.217 | <0.001 |
| Trouble with taste | -15.833 | 0.000 | 15.833 | -21.732 | -9.935 | <0.001 |
| Trouble swallowing saliva | -10.189 | 1.333 | 11.523 | -16.418 | -3.961 | 0.002 |
| Trouble with coughing | -21.417 | 7.333 | 28.750 | -28.634 | -14.200 | <0.001 |

Table 6.5: Welch 2-sided t-test of Mean Quality of Life Symptomatology Results Between Cancer and Live Donor Patients Reaching both Clinical and Statistical Significance *(continued)*

| Domain | Difference | Cancer | Live donor | Low CI | High CI | p-value |
|---|---|---|---|---|---|---|
| Weight Loss | -20.988 | 0.000 | 20.988 | -28.119 | -13.856 | <0.001 |

Table 6.6: Welch 2-sided t-test of Mean Quality of Life Symptomatology Results Between Vascular and Live Donor Patients Reaching both Clinical and Statistical Significance

| Domain | Difference | Vascular | Live donor | Low CI | High CI | p-value |
|---|---|---|---|---|---|---|
| Quality of life overall | 24.128 | 30.128 | 6.000 | 17.296 | 30.960 | <0.001 |
| Fatigue | 22.312 | 25.534 | 3.222 | 14.066 | 30.557 | <0.001 |
| Pain | 21.038 | 24.038 | 3.000 | 11.446 | 30.631 | <0.001 |
| Dyspnoea | 15.660 | 16.993 | 1.333 | 7.217 | 24.104 | <0.001 |
| Insomnia | 18.795 | 30.128 | 11.333 | 7.480 | 30.109 | 0.001 |
| Appetite Loss | 13.072 | 13.072 | 0.000 | 5.791 | 20.353 | 0.001 |
| Financial Difficulties | 16.340 | 16.340 | 0.000 | 7.083 | 25.597 | 0.001 |
| Eating | 10.577 | 10.577 | 0.000 | 4.176 | 16.978 | 0.002 |
| Pain and discomfort | 12.103 | 14.103 | 2.000 | 4.754 | 19.451 | 0.002 |
| Anxiety | 39.269 | 43.269 | 4.000 | 30.811 | 47.727 | <0.001 |
| Dry Mouth | 14.026 | 16.026 | 2.000 | 6.254 | 21.797 | 0.001 |
| Trouble with coughing | 12.538 | 19.872 | 7.333 | 5.516 | 19.560 | 0.001 |

Table 6.7: Welch 2-sided t-test of Mean Quality of Life Symptomatology Results Between Vascular and Cancer Patients Reaching both Clinical and Statistical Significance

| Domain | Difference | Cancer | Vascular | Low CI | High CI | p-value |
|---|---|---|---|---|---|---|
| Pain | 11.281 | 24.038 | 12.757 | 1.039 | 21.523 | 0.031 |
| Appetite Loss | -11.208 | 13.072 | 24.280 | -21.659 | -0.757 | 0.036 |
| Dysphagia | -17.543 | 2.457 | 20.000 | -24.189 | -10.897 | $<$0.001 |
| Eating | -17.618 | 10.577 | 28.194 | -26.398 | -8.837 | $<$0.001 |
| Odynophagia | -14.583 | 4.167 | 18.750 | -21.929 | -7.238 | $<$0.001 |
| Anxiety | -12.147 | 43.269 | 55.417 | -22.099 | -2.196 | 0.017 |
| Eating with others | -11.410 | 1.923 | 13.333 | -19.120 | -3.700 | 0.004 |
| Dry Mouth | -10.641 | 16.026 | 26.667 | -20.854 | -0.428 | 0.041 |
| Trouble with taste | -10.605 | 5.229 | 15.833 | -18.320 | -2.889 | 0.007 |
| Trouble swallowing saliva | -10.869 | 0.654 | 11.523 | -16.962 | -4.776 | 0.001 |
| Weight Loss | -16.500 | 4.487 | 20.988 | -25.064 | -7.937 | $<$0.001 |

### 6.3.3 Patient-reported Functional Domains

Functional domain data collected are presented in Figure 6.2. This reveals, as expected, that the majority of patients have generally preserved function. The LDN group have the most preserved function, with few reductions. Perhaps slightly surprisingly, the AAA patients scored lower in the functional domains than the UGIC patients. This could represent the relatively preserved character of the UGIC patients reaching resection as compared to the more advanced stage of the AAA patients who have reached criteria for repair.

Comparisons between patient types are presented in Table 6.10, Table 6.11, and Table 6.12. These reveal that, as expected, there are clinically and statistically significant differences between the LDN patients and both other patient types across all domains other than *"Physical Functioning"* between the LDN and the UGIC group, and *"Body Image"* between the LDN and the AAA group. In neither of these domains did the difference reach clinical significance. In the comparison between UGIC and AAA patients, *"Physical Functioning"* and *"Role Functioning"* were better preserved in the UGIC group than the AAA group. This may again represent the stage of disease at time of presentation for each group.

Testing within each group did not reveal any functional domain which reached both clinical and statistical significance across any CT-BCA muscle variable. For completion, examples from the UGIC are shown in Table 6.8 for SMI cut-points, and in Table 6.9 for SMD cut-points.

As described in Chapter 3, the cut-points used were those defined by Martin *et al* for SMI (High BMI Male $<53cm^2/m^2$, Normal BMI Male $<43cm^2/m^2$, High BMI Female $<41cm^2/m^2$, Normal BMI Female $<41cm^2/m^2$), and for SMD (High BMI Male <33HU, Normal BMI Male <41HU, High BMI Female <43HU, Normal BMI Female <41HU)[18].

Table 6.8: Welch 2-sided t-test of Mean Quality of Life Functionality Results Within UGIC Patients by Martin et al SMI Cut-Points

| Domain | Difference | Normal SMI | Low SMI | Low CI | High CI | p-value |
|---|---|---|---|---|---|---|
| Physical Functioning | -3.460 | 90.909 | 94.369 | -8.283 | 1.362 | 0.157 |
| Role Functioning | -2.818 | 86.822 | 89.640 | -12.712 | 7.076 | 0.572 |
| Emotional Functioning | -8.483 | 75.000 | 83.483 | -18.220 | 1.253 | 0.087 |
| Cognitive Functioning | -2.754 | 85.985 | 88.739 | -11.060 | 5.553 | 0.511 |
| Social Functioning | 2.795 | 85.227 | 82.432 | -8.488 | 14.078 | 0.623 |
| Body Image | -6.369 | 83.721 | 90.090 | -17.872 | 5.133 | 0.273 |

Table 6.9: Welch 2-sided t-test of Mean Quality of Life Functionality Results within UGIC Patients According to Martin et al SMD Cut-Points

| Domain | Difference | Normal SMD | Low SMD | Low CI | High CI | p-value |
|---|---|---|---|---|---|---|
| Physical Functioning | 0.267 | 92.632 | 92.364 | -4.764 | 5.299 | 0.916 |
| Role Functioning | -3.048 | 86.486 | 89.535 | -13.154 | 7.058 | 0.55 |
| Emotional Functioning | 1.513 | 79.678 | 78.165 | -8.890 | 11.916 | 0.772 |
| Cognitive Functioning | 0.898 | 87.719 | 86.822 | -7.460 | 9.256 | 0.831 |
| Social Functioning | -0.337 | 83.772 | 84.109 | -11.708 | 11.035 | 0.953 |
| Body Image | -8.020 | 82.456 | 90.476 | -20.323 | 4.283 | 0.197 |

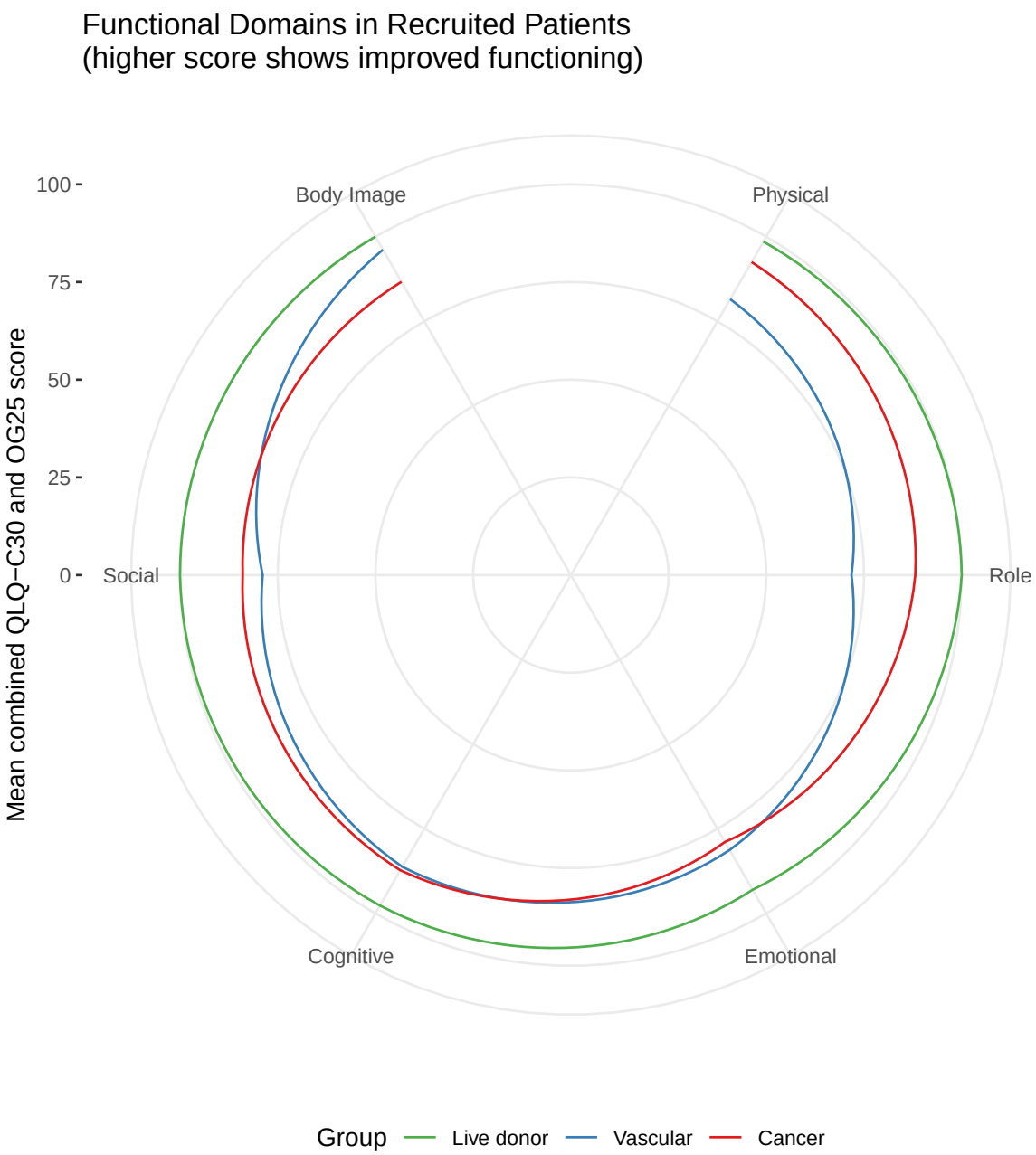



Figure 6.2: Quality of life Functional Domains by Patient Type

Table 6.10: Welch 2-sided t-test of Mean Quality of Life Functionality Results Between Live Donor and Cancer Patients Reaching both Clinical and Statistical Significance

| Domain | Difference | Live donor | Cancer | Low CI | High CI | p-value |
|---|---|---|---|---|---|---|
| Role Functioning | 11.875 | 100.000 | 88.125 | 6.896 | 16.854 | <0.001 |
| Emotional Functioning | 14.125 | 93.000 | 78.875 | 8.378 | 19.872 | <0.001 |
| Cognitive Functioning | 10.424 | 97.667 | 87.243 | 5.993 | 14.855 | <0.001 |
| Social Functioning | 16.049 | 100.000 | 83.951 | 10.357 | 21.742 | <0.001 |
| Body Image | 13.333 | 100.000 | 86.667 | 7.362 | 19.305 | <0.001 |

Table 6.11: Welch 2-sided t-test of Mean Quality of Life Functionality Results Between Live Donor and Vascular Patients Reaching both Clinical and Statistical Significance

| Domain | Difference | Live donor | Vascular | Low CI | High CI | p-value |
|---|---|---|---|---|---|---|
| Physical Functioning | 16.995 | 98.533 | 81.538 | 11.049 | 22.941 | <0.001 |
| Role Functioning | 28.205 | 100.000 | 71.795 | 18.243 | 38.167 | <0.001 |
| Emotional Functioning | 11.750 | 93.000 | 81.250 | 4.587 | 18.913 | 0.002 |
| Cognitive Functioning | 11.449 | 97.667 | 86.218 | 4.116 | 18.781 | 0.003 |
| Social Functioning | 21.154 | 100.000 | 78.846 | 13.460 | 28.848 | <0.001 |

Table 6.12: Welch 2-sided t-test of Mean Quality of Life Functionality Results Between Cancer and Vascular Patients Reaching both Clinical and Statistical Significance

| Domain | Difference | Cancer | Vascular | Low CI | High CI | p-value |
|---|---|---|---|---|---|---|
| Physical Functioning | -10.951 | 81.538 | 92.490 | -17.241 | -4.661 | 0.001 |
| Role Functioning | -16.330 | 71.795 | 88.125 | -27.396 | -5.265 | 0.004 |

### 6.3.4 Objective functioning

As an objective measure of physical functioning, gait speed measured in metres per second ($ms^{-1}$) was felt to be useful in line with the EWGSOP consensus [124]. This defines *"0.8ms$^{-1}$"* as a cut-point below which patient function can be said to have objectively deteriorated. The current cohort gait speed is presented in Figure 6.3 with colours and shapes representing CT-BCA, and in Table 6.13. As shown, there were ***3*** UGIC and ***2*** AAA patients in the whole cohort with gait speed under this $0.8ms^{-1}$ cut-point. Additionally, the distribution of patients above and below the CT-BCA cut-points does not appear to allow categorisation of patients.

Attempts to use linear regression to explore relationships between CT_BCA muscle variables and gait speed failed. There were no statistically significant relationships found between gait speed and either SMI or SMD. Narrowing the focus of the analysis to look at UGIC, AAA, and LDN groups did find a statistically significant relationship between gait speed and SMI in the LDN group ($p = 0.0482$), however the relationship was very weak ($R^2 = 0.073$). Subdividing the patient groups by sex revealed a weak relationship in the female UGIC group ($R^2 = 0.270$, $p = 0.011$).

Investigating SMD and gait speed revealed a weak relationship across the whole cohort ($R^2 = 0.057$, $p = 0.003$). However this disappeared when looking at individual patient groups; patient groups by sex; and a relationship was only seen in high BMI AAA males: though this was again weak ($R^2 = 0.13$, $p = 0.04$).

### 6.3.5 EQ5D-3L Questionnaire

Across all patient types, there were so few patients reporting anything other than *"normal"* responses to the EQ5D questionnaire that it rendered attempts at analysis futile.

Table 6.13: Gait Speed by Patient Type and Sex/BMI Subgroup

| Patient Type | Sex/BMI Subgroup | Min Gait Speed | Mean Gait Speed | Max Gait Speed |
|---|---|---|---|---|
| **Live donor** | | | | |
| | Female | 1.190 | 1.683 | 2.198 |
| | Low BMI Male | 1.230 | 1.650 | 2.490 |
| | High BMI Male | 1.105 | 1.535 | 1.935 |
| **Vascular** | | | | |
| | Female | 0.974 | 1.583 | 2.459 |
| | Low BMI Male | 0.948 | 1.206 | 1.835 |
| | High BMI Male | 0.389 | 1.293 | 1.729 |
| **Cancer** | | | | |
| | Female | 0.792 | 1.280 | 1.881 |
| | Low BMI Male | 0.796 | 1.514 | 1.967 |
| | High BMI Male | 0.619 | 1.493 | 2.055 |

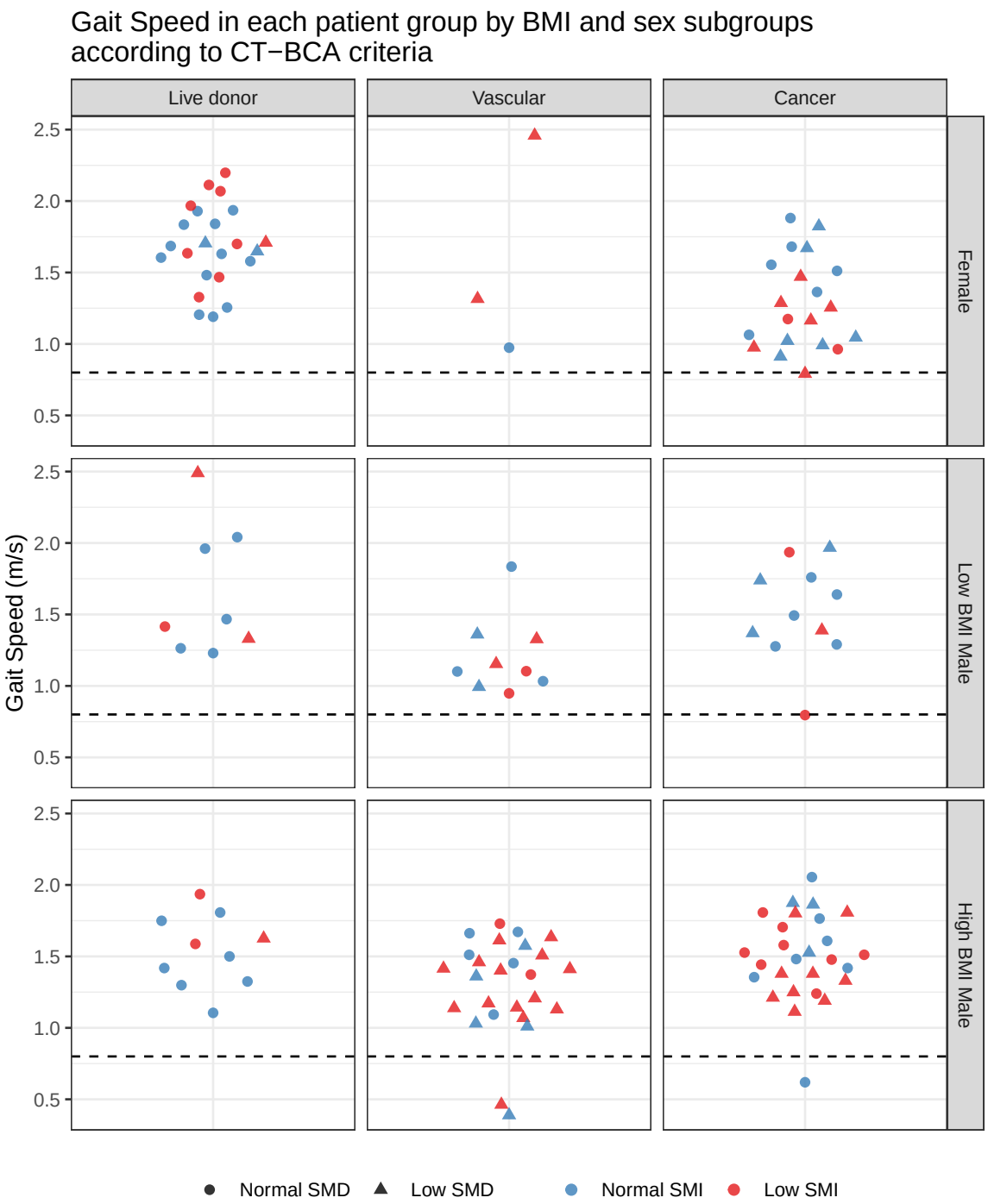



Figure 6.3: Objective Function (Gait Speed) by Group and BMI/Sex Subgroup According to CT-BCA Variable Cut-points

### 6.3.6 Performance Status

As noted in Chapter 1, there is agreement between patient-reported performance status and physician-assigned performance status [146]. What has not been reported is whether this performance status translates to mirror patient-reported QoL measures. To this end, both the symptomatology and functional domains were interrogated to ascertain how well performance status related to PROM responses. This was initially performed across the entire cohort, as seen in Table 6.14 for symptomatic and Table 6.15 for functional domains. As can be seen, there is statistical significance in the differences in score by ECOG status across the majority of domains. This is in keeping with higher performance status patients having lower symptomatology and better functionality, and supports the concordance between physician-assigned and patient-reported performance status.

When attempting to ascertin whether these differences held true in each patient group, it became clear that the distribution of patients across performance status was not equal. In each group there were reasonable numbers of ECOG 0 (LDN 49; AAA 23; UGIC 44) patients. Numbers of ECOG 1 patients were maintained in AAA (23) and UGIC (35) groups but not in the LDN (1) group. There were few ECOG 2 (LDN 0; AAA 2; UGIC 1) and ECOG 3 (LDN 0; AAA 4; UGIC 1) patients, and no ECOG 4 or higher patients. Given the paucity of lower performance status patients in each patient group, the per-group analysis was not performed.

Table 6.14: Patient-reported Symptomatic Domains by Performance Status

| Dependent: ECOG Score | | 0 | 1 | 2 | 3 | p |
|---|---|---|---|---|---|---|
| Quality of life overall | Mean (SD) | 13.9 (16.3) | 36.3 (21.6) | 55.6 (9.6) | 60.0 (32.5) | <0.001 |
| Fatigue | Mean (SD) | 8.1 (11.9) | 29.2 (26.7) | 63.0 (33.9) | 64.4 (28.8) | <0.001 |
| Nausea and Vomiting | Mean (SD) | 3.4 (9.5) | 10.7 (20.7) | 0.0 (0.0) | 33.3 (40.8) | <0.001 |
| Pain | Mean (SD) | 5.7 (12.6) | 21.5 (29.2) | 50.0 (50.0) | 70.0 (29.8) | <0.001 |
| Dyspnoea | Mean (SD) | 4.9 (13.4) | 14.7 (26.5) | 22.2 (38.5) | 20.0 (44.7) | 0.008 |
| Insomnia | Mean (SD) | 15.8 (23.5) | 31.6 (35.8) | 66.7 (33.3) | 66.7 (33.3) | <0.001 |
| Appetite Loss | Mean (SD) | 7.5 (19.3) | 22.0 (34.8) | 55.6 (38.5) | 60.0 (43.5) | <0.001 |
| Constipation | Mean (SD) | 7.2 (16.9) | 10.2 (20.8) | 33.3 (33.3) | 6.7 (14.9) | 0.092 |
| Diarrhoea | Mean (SD) | 3.7 (12.3) | 13.6 (22.4) | 0.0 (0.0) | 0.0 (0.0) | 0.002 |
| Financial Difficulties | Mean (SD) | 2.9 (13.6) | 13.2 (29.2) | 44.4 (50.9) | 60.0 (43.5) | <0.001 |
| Dysphagia | Mean (SD) | 4.9 (13.2) | 15.8 (28.7) | 27.8 (20.0) | 28.9 (36.5) | 0.001 |
| Eating | Mean (SD) | 9.6 (17.3) | 21.9 (29.4) | 44.4 (37.6) | 55.0 (48.4) | <0.001 |
| Reflux | Mean (SD) | 4.8 (10.3) | 16.1 (23.6) | 5.6 (9.6) | 36.7 (27.4) | <0.001 |
| Odynophagia | Mean (SD) | 6.4 (15.5) | 12.1 (27.1) | 16.7 (28.9) | 43.3 (43.5) | 0.001 |
| Pain and discomfort | Mean (SD) | 9.4 (19.4) | 14.7 (22.1) | 50.0 (44.1) | 40.0 (54.8) | <0.001 |
| Anxiety | Mean (SD) | 28.0 (31.4) | 54.2 (25.6) | 77.8 (19.2) | 46.7 (36.1) | <0.001 |
| Eating with others | Mean (SD) | 2.6 (14.1) | 11.3 (28.1) | 33.3 (57.7) | 20.0 (44.7) | 0.005 |
| Dry Mouth | Mean (SD) | 10.7 (19.0) | 26.0 (35.6) | 0.0 (0.0) | 60.0 (36.5) | <0.001 |
| Trouble with taste | Mean (SD) | 4.1 (13.4) | 14.4 (27.3) | 33.3 (33.3) | 26.7 (43.5) | <0.001 |
| Trouble swallowing saliva | Mean (SD) | 1.7 (7.4) | 13.8 (30.6) | 0.0 (0.0) | 6.7 (14.9) | 0.001 |
| Choked when Swallowing | Mean (SD) | 3.2 (13.2) | 7.9 (21.7) | 0.0 (0.0) | 0.0 (0.0) | 0.266 |
| Trouble with coughing | Mean (SD) | 18.8 (21.2) | 24.3 (29.6) | 22.2 (19.2) | 6.7 (14.9) | 0.305 |
| Trouble Talking | Mean (SD) | 0.3 (3.1) | 5.6 (14.1) | 0.0 (0.0) | 6.7 (14.9) | 0.001 |
| Weight Loss | Mean (SD) | 6.3 (18.6) | 16.9 (31.8) | 0.0 (0.0) | 40.0 (43.5) | 0.002 |
| Hair Loss | Mean (SD) | 0.7 (5.0) | 7.2 (14.1) | 0.0 (NA) | 11.1 (19.2) | 0.032 |

Table 6.15: Patient-reported Functionality Domains by Performance Status

| Dependent: ECOG Score | | 0 | 1 | 2 | 3 | p |
|---|---|---|---|---|---|---|
| Physical Functioning | Mean (SD) | 96.8 (7.2) | 83.6 (16.7) | 55.6 (27.8) | 65.3 (28.8) | <0.001 |
| Role Functioning | Mean (SD) | 96.7 (13.6) | 73.2 (30.3) | 44.4 (50.9) | 43.3 (46.5) | <0.001 |
| Emotional Functioning | Mean (SD) | 87.7 (17.3) | 76.9 (23.3) | 63.9 (33.7) | 73.3 (43.5) | 0.003 |
| Cognitive Functioning | Mean (SD) | 94.7 (10.2) | 83.1 (24.9) | 66.7 (44.1) | 70.0 (41.5) | <0.001 |
| Social Functioning | Mean (SD) | 96.1 (10.8) | 73.4 (29.1) | 55.6 (50.9) | 50.0 (40.8) | <0.001 |
| Body Image | Mean (SD) | 97.4 (11.8) | 87.6 (25.4) | 88.9 (19.2) | 60.0 (54.8) | <0.001 |

## 6.4 Discussion of CT-BCA Variable Relationship with QoL Metrics

### 6.4.1 Current QoL PROMs In Pre-Operative Patients

There is increasing interest in the use of PROMs to help define trial end-points and to assess patient quality of life through the healthcare journey. Many of these PROMs have been validated in cancer patients, though the patients used for validation of the PROM used in the current study were from a non-resectable lung cancer cohort [172]. When considering the PROM outcomes recorded in the present study, this would seem to fit with the symptomatology and reported functionality. Reporting instructions recommend a difference between groups of 10 points to reach clinical significance. This was rarely seen, as the majority of the patients under study had little symptomatology and good reported function. From this, it could perhaps be suggested that the sensitivity of these instruments for detecting relevant QoL changes in a relatively well-preserved pre-operative cohort is limited.

### 6.4.2 Ability of CT-BCA to Subdivide Current PROMs

The results noted above show that the symptoms and function reported by patients appears relatively unaffected by CT cut-points in the current cohort. This may be related to the suggestion than the translation of CT-BCA variables to patient symptomatology and reported function is non-linear. There is, within the literature, a recognition that different populations across the globe will have different distributions for SMI and potentially for SMD. It is known, for example, that South East Asian patients have a lower SMI than those of a western European background [125].

What is also important to note, is that cut-points derived from a western European and North American population may not maintain their relevance even within that population [107]. The SMI and SMD distribution of patients shown in Chapter 5 gives weight to this suggestion, and it may be that each population under study will accordingly need bespoke cut-points identified. The choice of population to use for such cut-point identification studies is difficult: it is currently unusual for healthy individuals to undergo CT scanning; taking cut-points from the population under study may be confounded by disease processes; and if studies are multi-centre, cut-points derived at one centre may not be applicable to the other centres. Nevertheless, the utility and repeated demonstration of prognostic value derived from published cut-points in differing populations lends credence to their use in signposting disease progression.

### 6.4.3 Ability of CT-BCA to Subdivide Objective Measures of Function

By their nature, patients recruited from a preoperative cohort must be sufficiently fit to undergo their planned procedure. It is unusual in Scotland (and very possibly unethical) to offer surgery to those who are likely to succumb during or immediately following surgery. Accordingly, those patients considered for operative intervention will have sufficiently preserved physical function as to enable them to undergo major surgery. Additionally, those considered *"borderline"* for such a procedure are assessed by multiple members of the peri-operative team and may be declined for surgery if felt unfit. With this in mind, it is not unexpected to find very few patients in the current study falling below the EWGSOP functional cut-point of a gait speed less than $0.8ms^{-1}$. Whilst it would be intellectually pleasing to find CT-BCA variables neatly divided along objective functional lines, this is not borne out by the data. The spread of patients with normal and low SMI and SMD across the gait speed domain suggests that there is at best a limited relationship between CT-BCA and gait speed.

Thus patients can have defined cachexia; or CT-BCA sarcopenia; or both, and still exhibit preserved physical functioning. This finding seems to go against the accepted definition of cachexia as leading to *"progressive physical impairment"*, although it could easily be that the patients in the current study are in fact in the *"pre-cachexia"* stage of the process. This would suggest that the definition of cachexia, or the cut-points on which it is based may need to be revisited.

# Chapter 7

# Relationship Between CT Body Composition Variables and Muscle Protein Content

The relationship between skeletal muscle protein content of surgical biopsies and CT-BCA variables derived from patients submitting these biopsies is currently unclear. As discussed in Chapter 1, CT-BCA is being used as a trial outcome measure, and one assumption is that changes in CT-BCA (SMI and SMD) reflect changes in muscle biochemistry and specifically protein content. Additionally, the *reference* values for muscle protein content were derived from a small cohort of non-phenotyped American male trauma victims from the 1950s. The relationship of these individuals to, and how closely they represent modern European patients is unknown, as is the difference between *healthy (LDN)*, *non-cancer (AAA)*, and *cancer (UGIC)* patient muscle protein content.

We aimed to define *normal* as measured by modern copper reduction protein analytic techniques, and to relate this to *UGIC* and *AAA* groups.

## 7.1 Hypothesis and Research Questions

### 7.1.1 Hypothesis

There is a strong relationship between CT-BCA variables and skeletal muscle protein content.

### 7.1.2 Research Questions

- What is the skeletal muscle protein content of rectus abdominis muscle in the current study patients?
- Is there a difference in protein content between patient types?
- How does skeletal muscle volume relate to muscle protein content?
- How does skeletal muscle radio-density relate to muscle protein content?

## 7.2 Methods for Investigating CT and Protein Content Relationship

Patients undergoing surgical resection for upper gastrointestinal cancer (UGIC) (22 gastric, 70 oesophageal, 24 pancreatic and 4 with other upper GI cancers) were recruited. This group includes those patients previously recruited as part of another study. Additionally, non-cancer vascular aortic (AAA) (n=52) and healthy live kidney donor (LDN) (n=53) patients were recruited. Patients were defined as cachectic if they met the 2011 consensus definition criteria (as described in Chapter 1) [12]. Sarcopenia was defined using SMI cut-offs according to BMI [18]. SMD was measured between -30 and 150 HU, with low SMD defined as $<$41 HU with BMI $<$25 and $<$33 with BMI $>$25 [18]. Preoperative staging CT scans were analysed at L3 level using semi-automated Slice-O-Matic software v4.2 (Tomovision Montreal-Canada), which defines area measurements of skeletal muscle, subcutaneous fat, and visceral fat. Under general anaesthesia during resectional surgery, rectus abdominis muscle biopsies were taken, snap-frozen in liquid nitrogen, and stored at -80°C in monitored freezers prior to analysis. These biopsies were pulverized and weighed using an analytical balance (Mettler Toledo), then lysed with Phosphosafe Extraction Reagent (Merck Millipore, Burlington, Mass., USA) before being homogenized and centrifuged. The supernatant was analysed for total soluble protein (including myofibrillar and cystosolic) content using commercially available BCA protein assay kits (Pierce Biotechnology, Thermo Fisher Scientific, Rockford, IL) [66].

Statistics including linear modelling, and Welch 2-sided t-tests; and plotting were performed in R [168], as dicussed in Chapter 3.

### 7.2.1 Missing data analysis

An analysis was conducted with regard to missing data. As discussed in Chapter 3, the protein content analysis was conducted at a professional laboratory, and subject to the analysis priorities of this laboratory. As such, some of the samples were not analysed by the end of the study. Accordingly, there were cases in which protein content was not available for inclusion. A comparison between cases with and without protein content was undertaken to ascertain whether there was a significant difference in protein content with regard to potential explanatory variables. This revealed statistically significant differences in age (64.7 vs 60.4 years, $p = 0.009$), TUG (9.4 vs 8.6 seconds, $p = 0.03$), and gait speed (1.3 vs 1.6 m/s, $p < 0.001$) between patients with and without protein content analysis. A further analysis suggested that the pattern of missingness would support the decision to perform whole-case analysis as described in Chapter 3.

Table 7.1: Protein Content (microg/mg wet weight) overall

| Min | Max | Mean (sd) |
|---|---|---|
| 30.99 | 335.4 | 125.88 (72.74) |

Table 7.2: Protein Content (microg/mg wet weight) by Patient Type

| Patient Type | Min | Max | Mean (sd) |
|---|---|---|---|
| Live donor | 44.45 | 335.4 | 126.76 (74.77) |
| Vascular | 42.4 | 312.96 | 140.05 (86.09) |
| Cancer | 30.99 | 314.82 | 120.7 (67.17) |

## 7.3 Results for CT and Muscle Protein Content

### 7.3.1 Overall Protein Content

Across the entire cohort, mean muscle protein content was 125.88 (range 30.99-335.4) microg/mg wet weight. In each group, the mean (range) protein content was: LDN 126.76 (44.45-335.4); AAA 140.05 (42.4-312.96); UGIC 120.7 (30.99-314.82) microg/mg wet weight. These are shown in Table 7.1 and Table 7.2. Further segregation of muscle protein content by patient diagnosis, by sex, and by SMD are shown in Table 7.3. The overall protein content in this patient cohort is shown in Figure 7.1, and shows a normal distribution across all patient groups.

Table 7.3: Protein Content (microg/mg wet weight) by Patient Type, Sex, and SMD

| Patient Type | Sex | SMD | Min | Max | Mean (sd) |
|---|---|---|---|---|---|
| **Live donor** | | | | | |
| | Male | Normal | 59.59 | 303 | 123.85 (71.8) |
| | Male | Low | 61.29 | 61.94 | 61.61 (0.46) |
| | Female | Normal | 44.45 | 335.4 | 140.05 (80.62) |
| | Female | Low | - | - | - (-) |
| **Vascular** | | | | | |
| | Male | Normal | 42.4 | 300.33 | 112.09 (73.4) |
| | Male | Low | 62.2 | 296.06 | 162.95 (85.78) |
| | Female | Normal | 84.12 | 96.72 | 88.76 (6.92) |
| | Female | Low | 62.63 | 312.96 | 167.24 (121.72) |
| **Cancer** | | | | | |
| | Male | Normal | 50.99 | 314.82 | 131.32 (73.45) |
| | Male | Low | 30.99 | 271.43 | 120.24 (66.54) |
| | Female | Normal | 54.4 | 168.74 | 100.36 (33.45) |
| | Female | Low | 31.84 | 250.95 | 104.95 (73.3) |

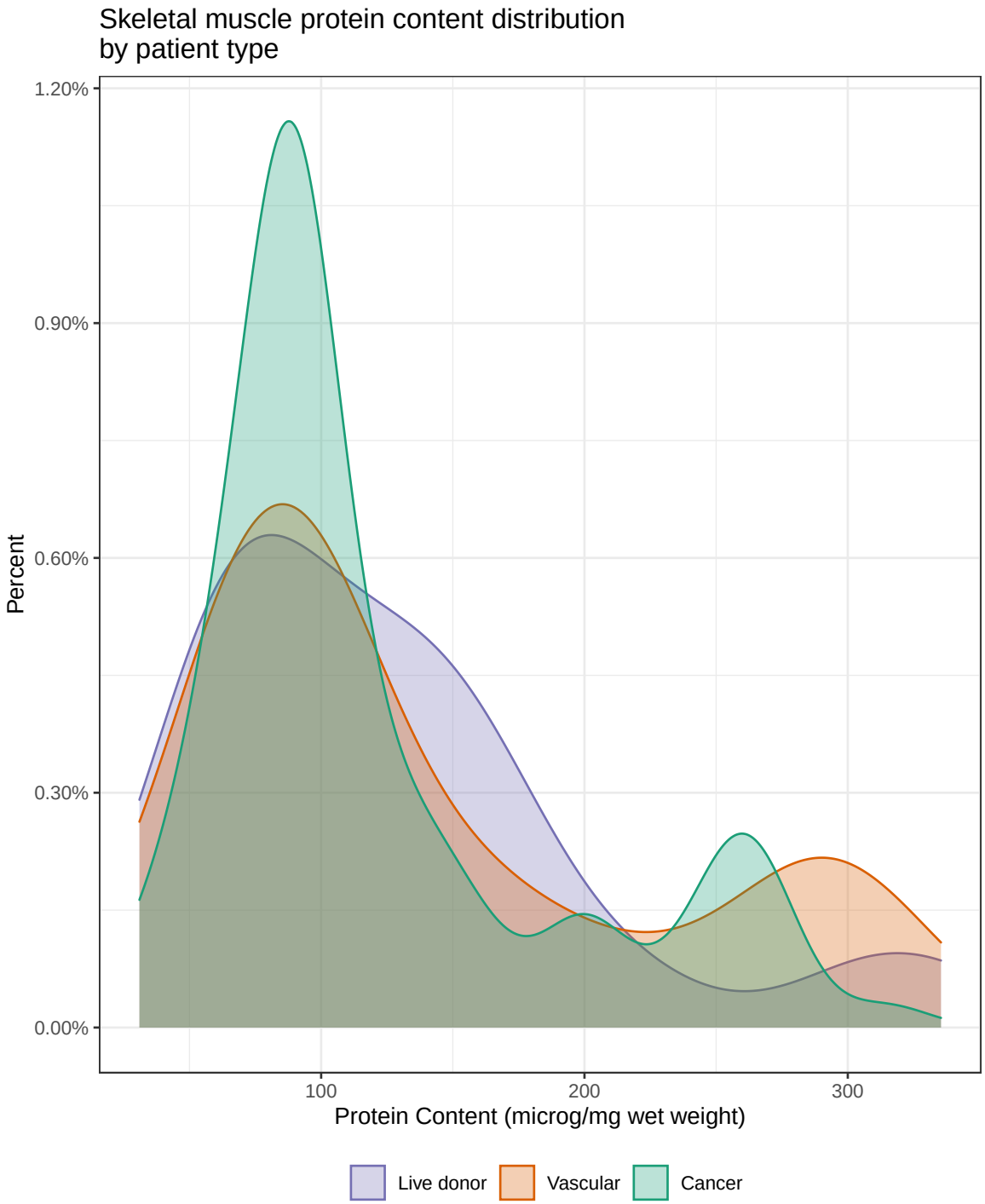



Figure 7.1: Skeletal Muscle Protein Content by Patient Type

Table 7.4: Welch 2-sided t-test of Muscle Protein Content in Normal vs Low SMD by Patient Type

| Group | Difference | Normal SMD | Low SMD | CI (low) | CI (high) | p value |
|---|---|---|---|---|---|---|
| Live donor | 71.071 | 132.685 | 61.614 | 37.644 | 104.498 | <0.001 |
| Vascular | -57.435 | 106.703 | 164.138 | -115.353 | 0.483 | 0.052 |
| Cancer | 7.820 | 124.036 | 116.217 | -20.973 | 36.612 | 0.59 |

### 7.3.2 Relationship Between CT-BCA Variables and Skeletal Muscle Protein Content

Applying the Martin *et al* [18] SMI cut-points to divide patients into *normal* and *low* SMI did not reveal any statistically significant differences in muscle protein content across any of the patient types. This can be seen graphically in Figure 7.2, showing that the spread of skeletal muscle protein content does not appear to diverge according to SMI cut-points.

Applying the Martin *et al* SMD cut-points to the same patients was expected to produce more of a difference as radio-density had been thought to bear more of a relationship with skeletal muscle constituent parts [59]. This did show some differences, as shown in Table 7.4, however it has to be noted that there were few patients in the *low SMD* LDN subgroup.

This is perhaps best illustrated in graphical form as seen in Figure 7.3 which shows that the spread of protein content is not obviously segregated by clinically-derived SMD cut-points.

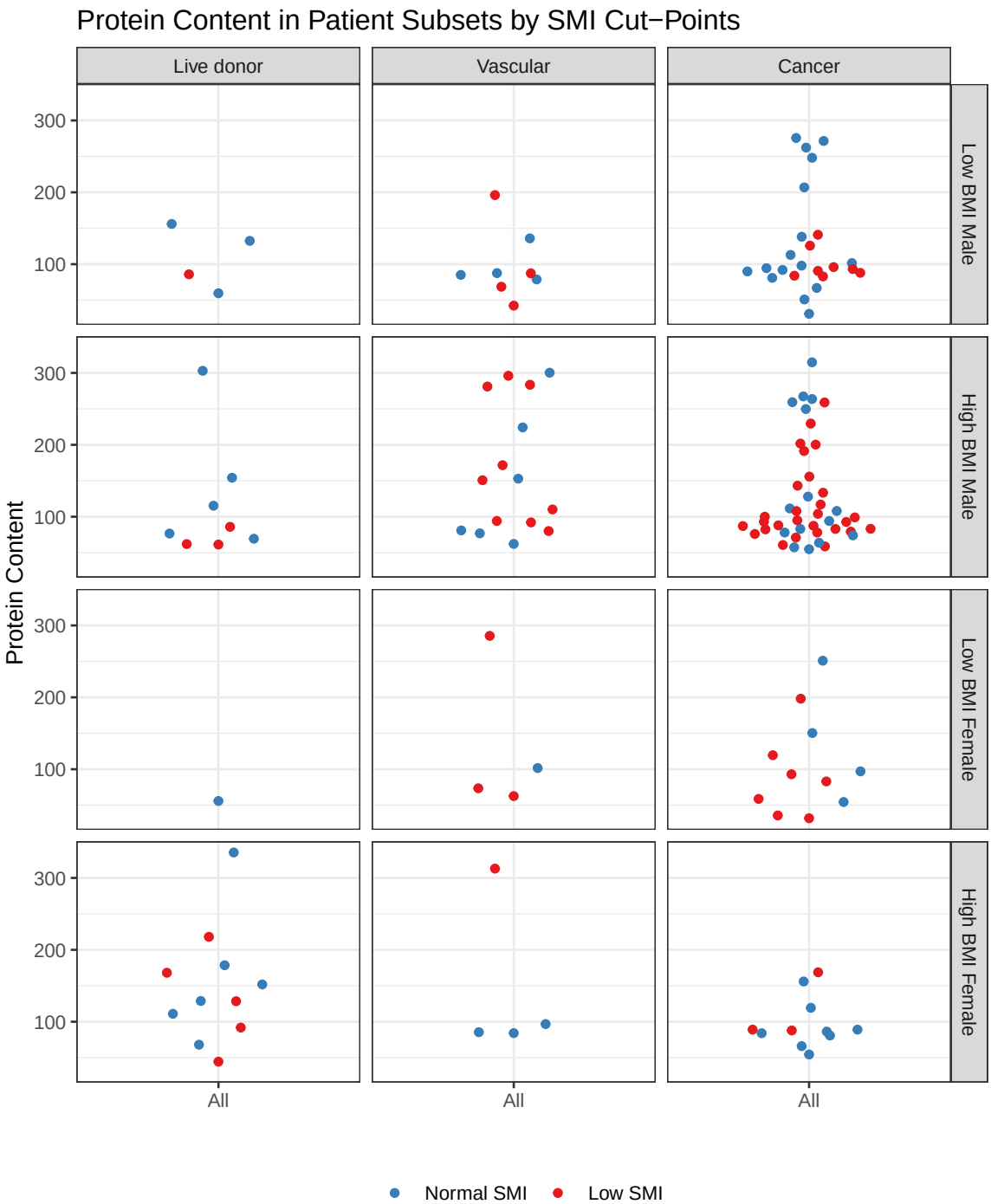



Figure 7.2: Protein Content in microg/mg wet weight by Patient Group and SMI

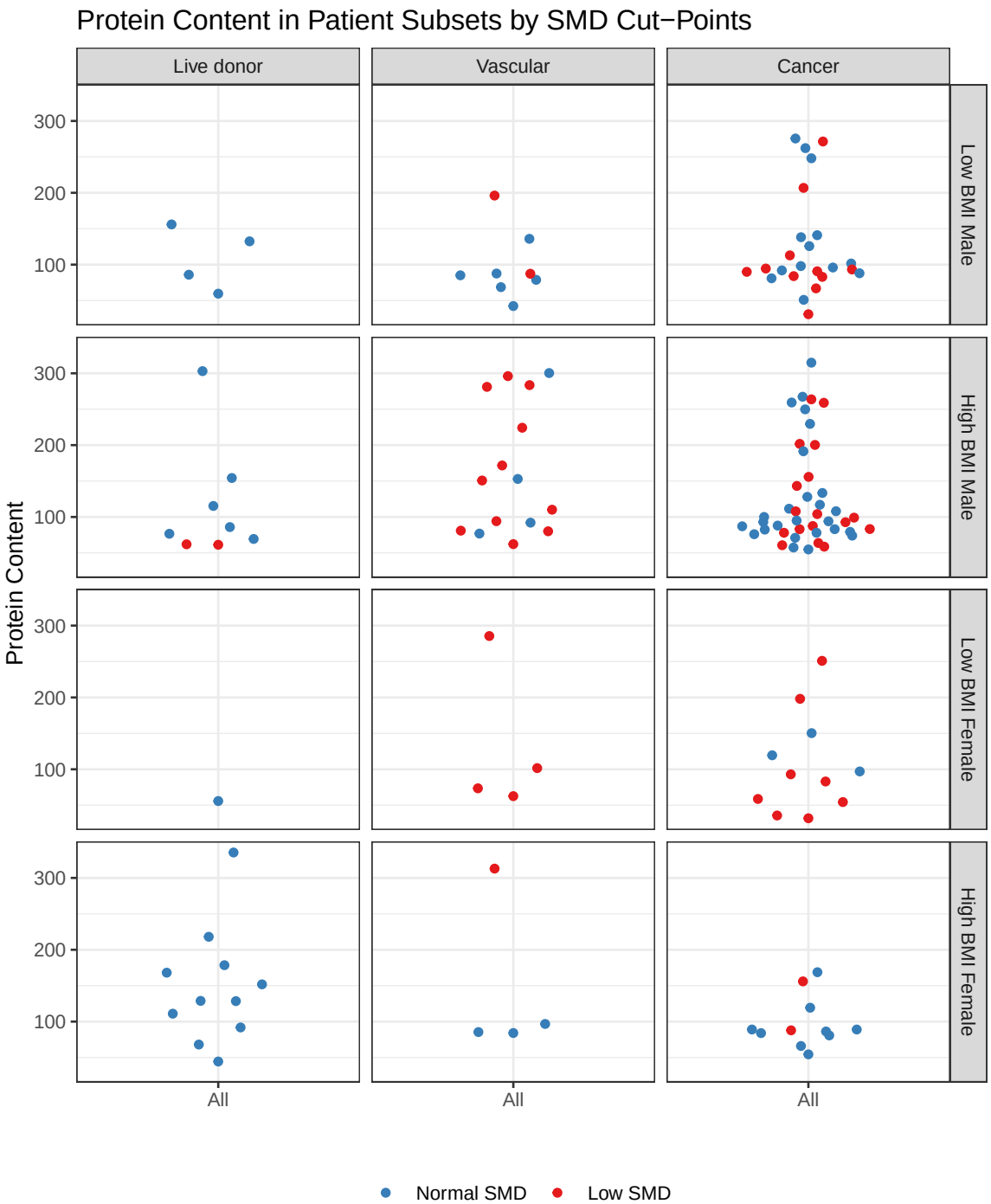



Figure 7.3: Protein Content in microg/mg wet weight by Patient Group and SMD

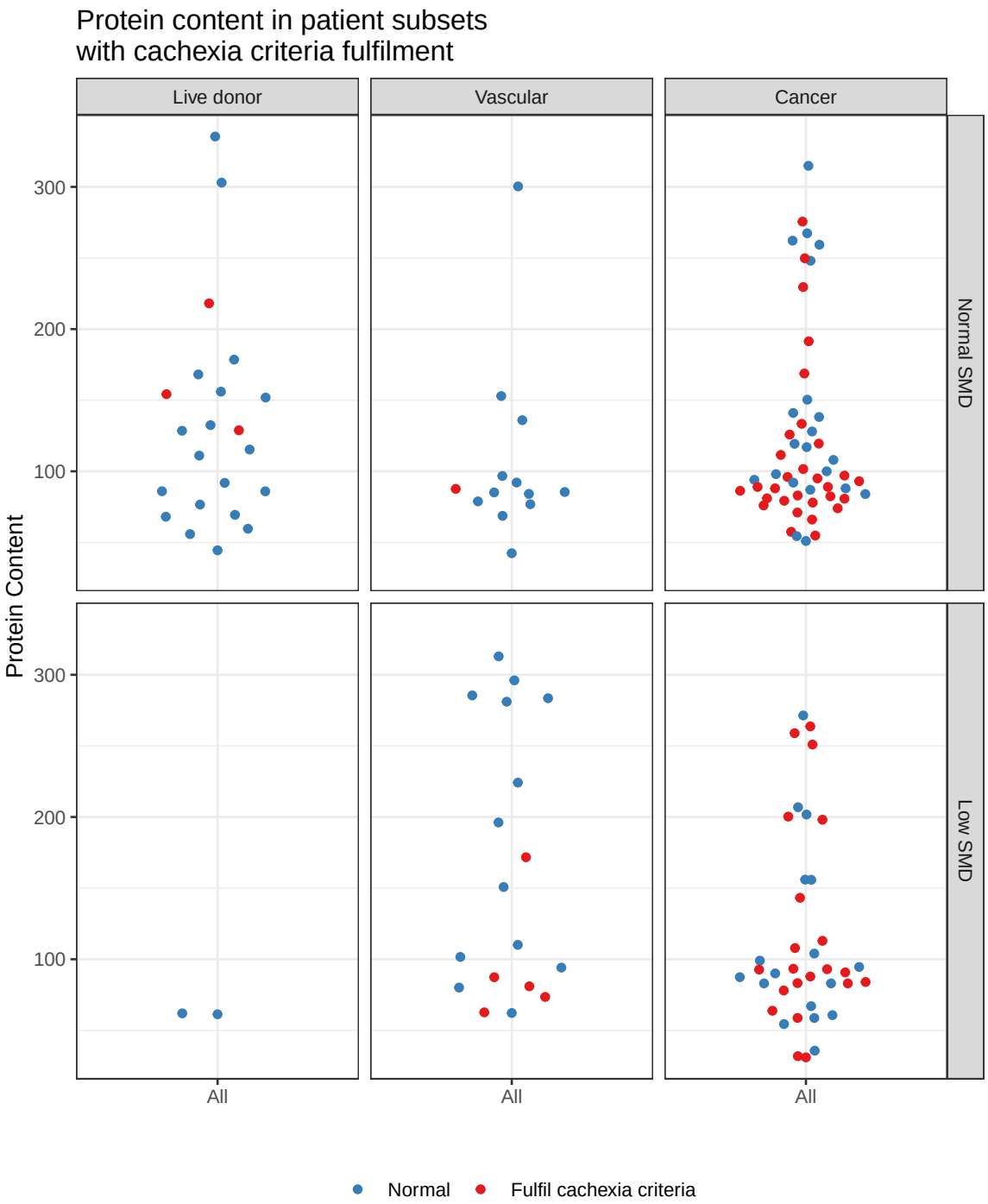



Figure 7.4: Protein Content in microg/mg wet weight by Patient Group With Regard to Cachexia

Interestingly, if the same data are plotted with regard to whether the patients fulfil the international consensus definition of cancer cachexia [12], the protein content distribution is that shown in Figure 7.4. It is worth noting that this is a specifically *"cancer"* cachexia definition, that the LDN population weight loss was voluntary, and that the AAA population do not have a recognised wasting disease despite their weight loss being involuntary. Nevertheless, it is important to note that in these and the cancer group, once again clinical definitions which *should* have a bearing on muscle composition do not appear to be usable to segregate patients into higher or lower muscle protein content subsets.

It may be that dichotomising data to try to find differences would require more data points than currently available to reach significance. In this case, it may be that relationships are present, and this should be investigated. Accordingly, linear regression was performed to ascertain the presence or otherwise of a relationship between CT-BCA variables and muscle protein content. These analyses were performed for each patient group overall, and then within each group by sex and BMI as appropriate. The investigations revealed no statistically significant relationships between SMD and skeletal muscle protein content, and are thus neither tabulated nor plotted.

There were statistically significant relationships between protein content and SMI in all patients, in cancer patients in general, and between protein content and SMI in high BMI male cancer patients within the cancer group as detailed in Tables 7.5 ($R^2 = 0.0324$, $p = 0.028$), 7.6 ($R^2 = 0.07$, $p = 0.012$ for UGIC, others non-significant), and 7.7 ($R^2 = 0.125$, $p = 0.02$ in high BMI male UGIC, others non-significant). It is to be noted, however, that the relationships are very weak, as shown in Figures 7.5, 7.6, and 7.7. The non-significant UGIC relationships were not plotted. The weakness of the relationships reaching statistical significance leads to the inference that although there is a potential relationship between CT-BCA variables and protein content of skeletal muscle, it is unclear that changes in CT-BCA can be relied upon or interpreted to reflect changes in muscle protein content during an intervention trial.

Table 7.5: Linear Regression between Protein Content and Skeletal Muscle Index

| r-squared | p value |
|---|---|
| 0.034 | 0.028 |

Table 7.6: Linear Regression between Protein Content and Skeletal Muscle Index

| Group | r-squared | p value |
|---|---|---|
| Live donor | 0.001 | 0.900 |
| Vascular | 0.013 | 0.537 |
| Cancer | 0.070 | 0.012 |

Table 7.7: Linear Regression between Protein Content and Skeletal Muscle Index in Upper GI Cancer patients

| Group | r-squared | p value |
|---|---|---|
| Female | 0.001 | 0.905 |
| Low BMI Male | 0.004 | 0.765 |
| High BMI Male | 0.125 | 0.020 |

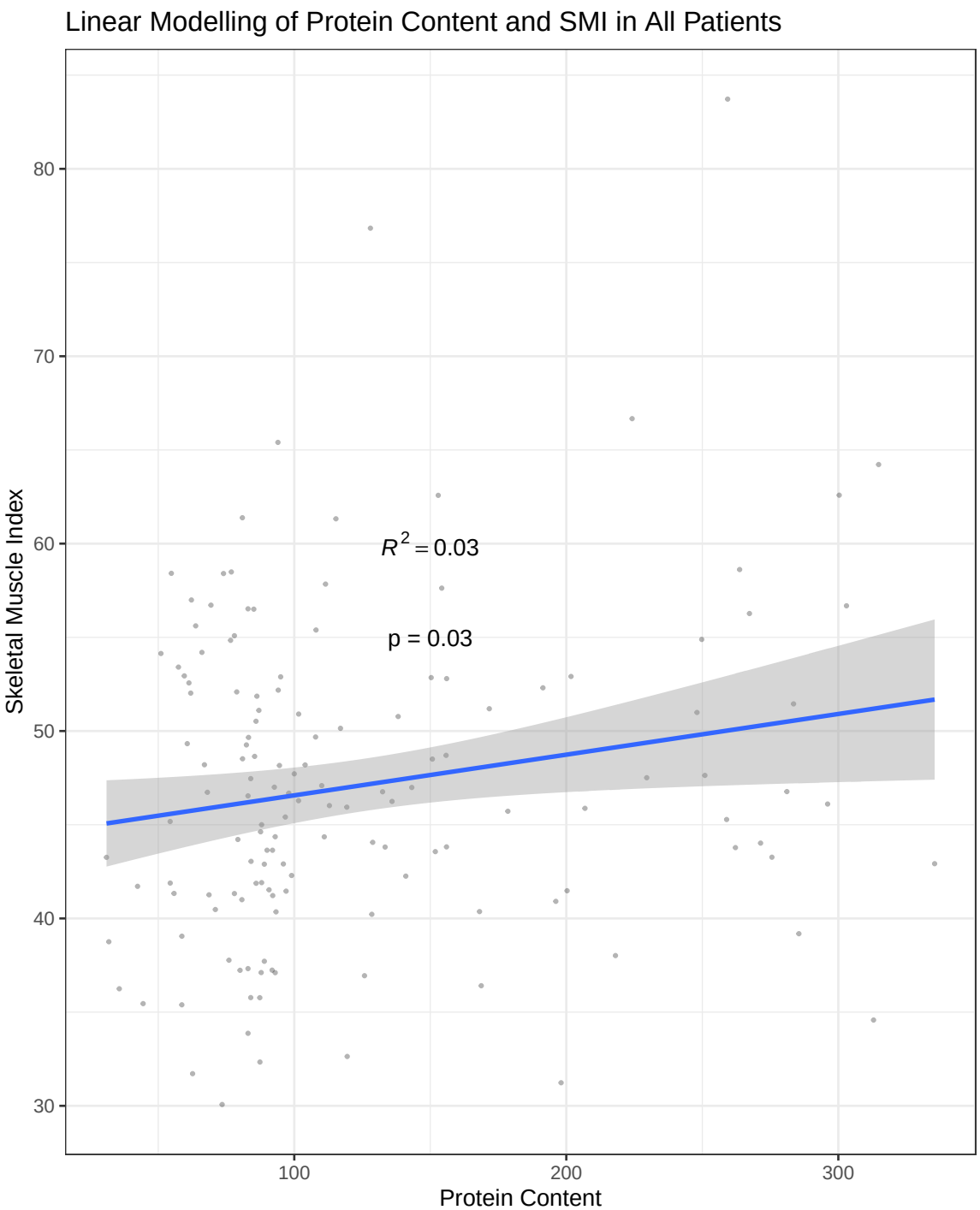



Figure 7.5: Linear Modelling of Protein Content and SMI in All Patients

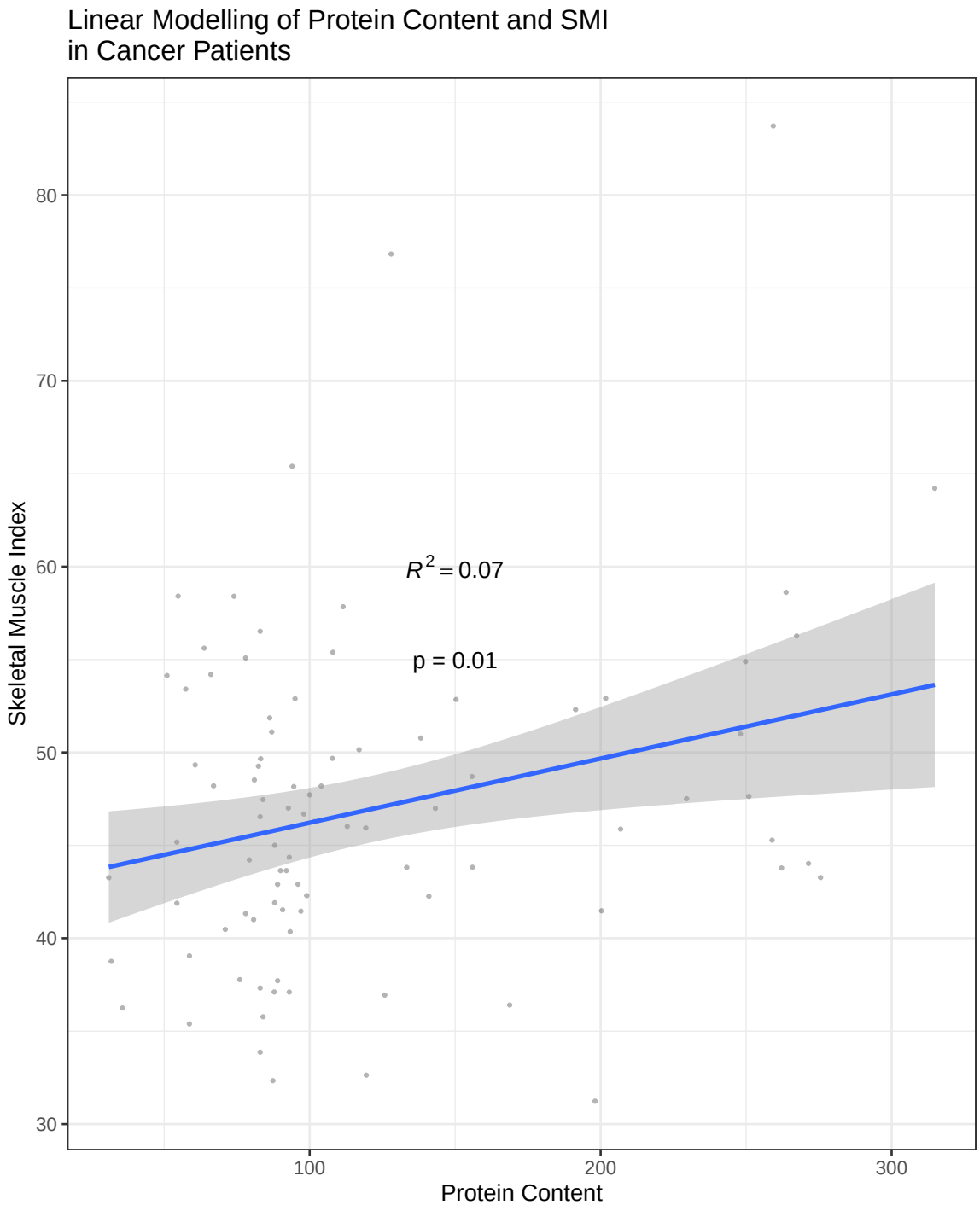



Figure 7.6: Linear Modelling of Protein Content and SMI in Cancer Patients

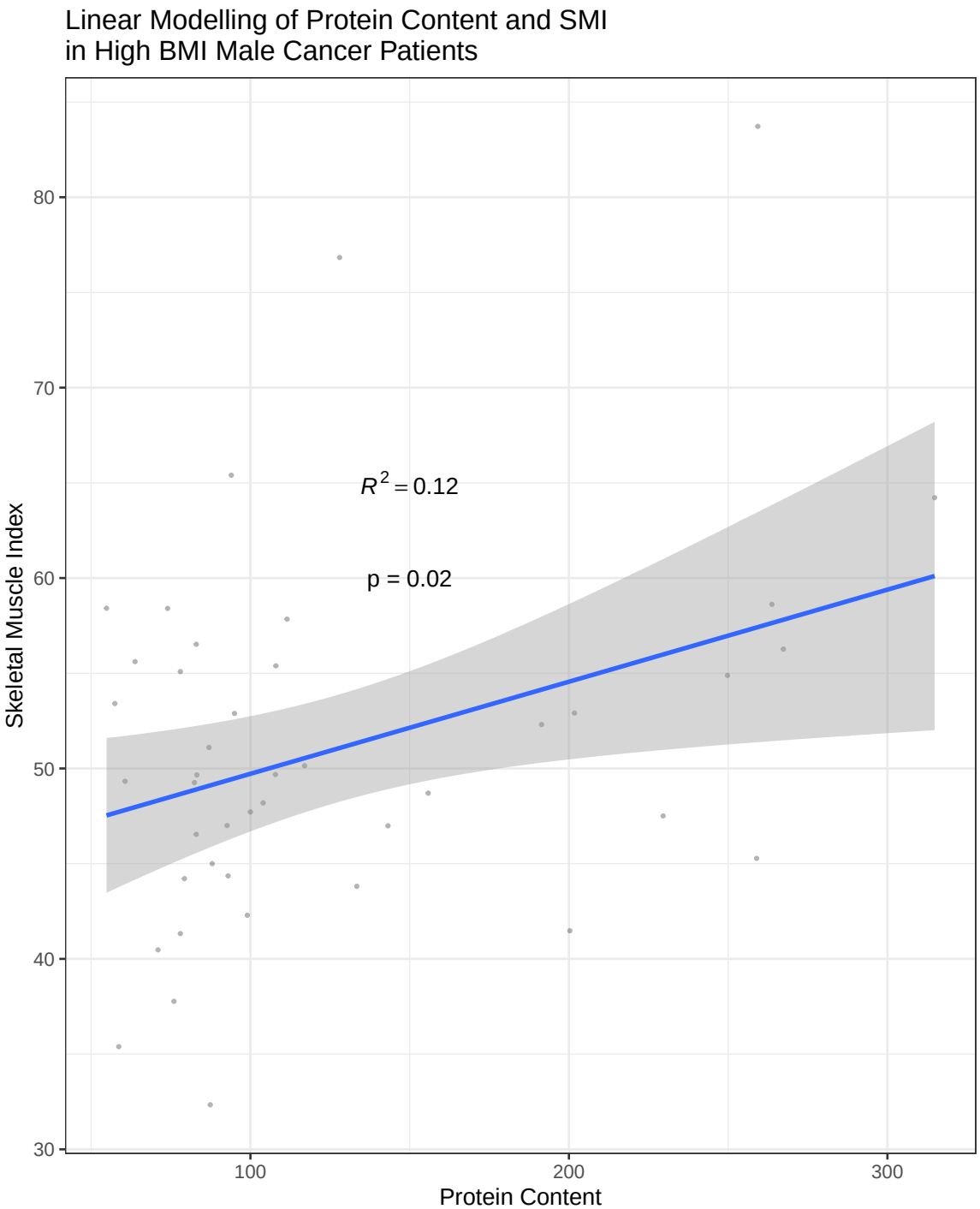



Figure 7.7: Linear Modelling of Protein Content and SMI in Male High BMI Cancer Patients

### 7.3.3 Multivariable Analysis of Protein Content

A multivariable model relating to skeletal muscle protein content was constructed as described in Chapter 3. This found that the predictors of muscle protein content were: Age, Gait Speed, and BMI. Of note, during the stepwise regression process, SMI was initially included in the model, but subsequently removed as having insufficient effect on the model once all of the above variables were included. This model is shown in Table 7.8. The Akaike information criterion for this model, however, remains high suggesting that the model is not well fitted. Additionally, for this model whole case analysis was performed, and as described above some of the UGIC patients were recruited in an earlier study. This previous study did not collect as much detailed phenotyping data as the more recent recruitment process; specifically gait speed was not measured. This reduces the number of patients able to undergo whole case analysis in each group, with only 13 LDN, 18 AAA, and 29 UGIC patients suitable for inclusion.

An exploratory investigation of multivariable modelling excluding gait speed as a potential explanatory variable was performed. This returned SMI, age, and whether the patient had lost weight as candidate variables. Comparing the 2 models revealed that although there were more cases to include, the fit of the second model (SMI + age + weight loss) was much worse than that of the first model (age + gait speed + BMI).

It appears that the multivariable model mirrors the findings of the univariable analysis, with limited support for any hypothesis relating CT-BCA variables to skeletal muscle protein content.

Table 7.8: Multivariable Modelling of Predictors of Muscle Protein Content

| Dependent: Protein Content | | unit | value | Coefficient (univariable) | Coefficient (multivariable) |
|---|---|---|---|---|---|
| Age | [29.0,90.0] | Mean (sd) | 125.9 (72.7) | 0.85 (-0.19 to 1.89, p=0.108) | 1.09 (-0.74 to 2.92, p=0.239) |
| Gait Speed (m/s) | [0.4,2.5] | Mean (sd) | 125.9 (72.7) | 62.92 (-2.86 to 128.70, p=0.060) | 82.71 (18.34 to 147.09, p=0.013) |
| BMI | [15.0,48.0] | Mean (sd) | 125.9 (72.7) | 1.86 (-0.42 to 4.14, p=0.108) | 5.20 (1.20 to 9.20, p=0.012) |
| Number in dataframe = 226, Number in model = 60, Missing = 166, Log-likelihood = -343.35, AIC = 696.7, R-squared = 0.17, Adjusted R-squared = 0.13 | | | | | |

### 7.3.4 Defining *"Normal"* and *"Low"* Skeletal Muscle Protein Content

In order to ascertain the possible effect of muscle protein content on CT-BCA and other clinical variables, it was decided to derive a muscle protein content cut-point of 1 standard deviation (SD) below the mean of the healthy LDN group to allow segregation of patients into those with *"Normal"* and *"Low"* protein content. Whilst in Chapter 5 a cut-point of 2 SD below the mean was used to define *"normal"*, when this technique was applied to protein content, there were no patients with protein content below the cut-point, and so this was felt to be of little worth.

The mean protein content of the LDN group was 126.76 microg/mg wet weight, with a standard deviation of 74.77. This gave a *normal/low* cut-point of 51.99 microg/mg wet weight, below which it could be said that patients had *"Low"* muscle protein content. Applying this cut-point to the study population, however, revealed that 1 LDN, 1 AAA, and 4 UGIC patients fell into the *"Low"* category. Clearly this definition is too restrictive, however it is currently unclear what level of protein content should be chosen instead.

### 7.3.5 Relationship Between Skeletal Muscle Protein Content and Physical Performance

Having investigated the relationship between CT-BCA and skeletal muscle protein content, the next step was to investigate whether measurable biochemical composition of muscle related to objective measures of physical performance. As described in the Methods section, timed-up-and-go tests were performed at the point of recruitment, with gait speed being captured as a part of this test. This allowed 2 distinct components to be analysed, capturing different aspects of patient physical functioning.

The analysis overall between gait speed and protein content did not reveal a statistically significant relationship, with R-squared value at 0.0594 and p=0.061. Investigating further, however, and analysing patient groups in isolation revealed a relationship reaching statistical significance for vascular patients as shown in Table 7.9, and in Figure 7.8.

Indexing gait speed for height, as described by Hof [173] and Stansfield *et al* [174] did not reveal any new significant relationships between protein content and indexed gait speed.

Table 7.9: Linear Modelling of Protein Content and Gait Speed in All Patients

| Group | r-squared | p value |
|---|---|---|
| Live donor | 0.003 | 0.859 |
| Vascular | 0.220 | 0.050 |
| Cancer | 0.009 | 0.619 |

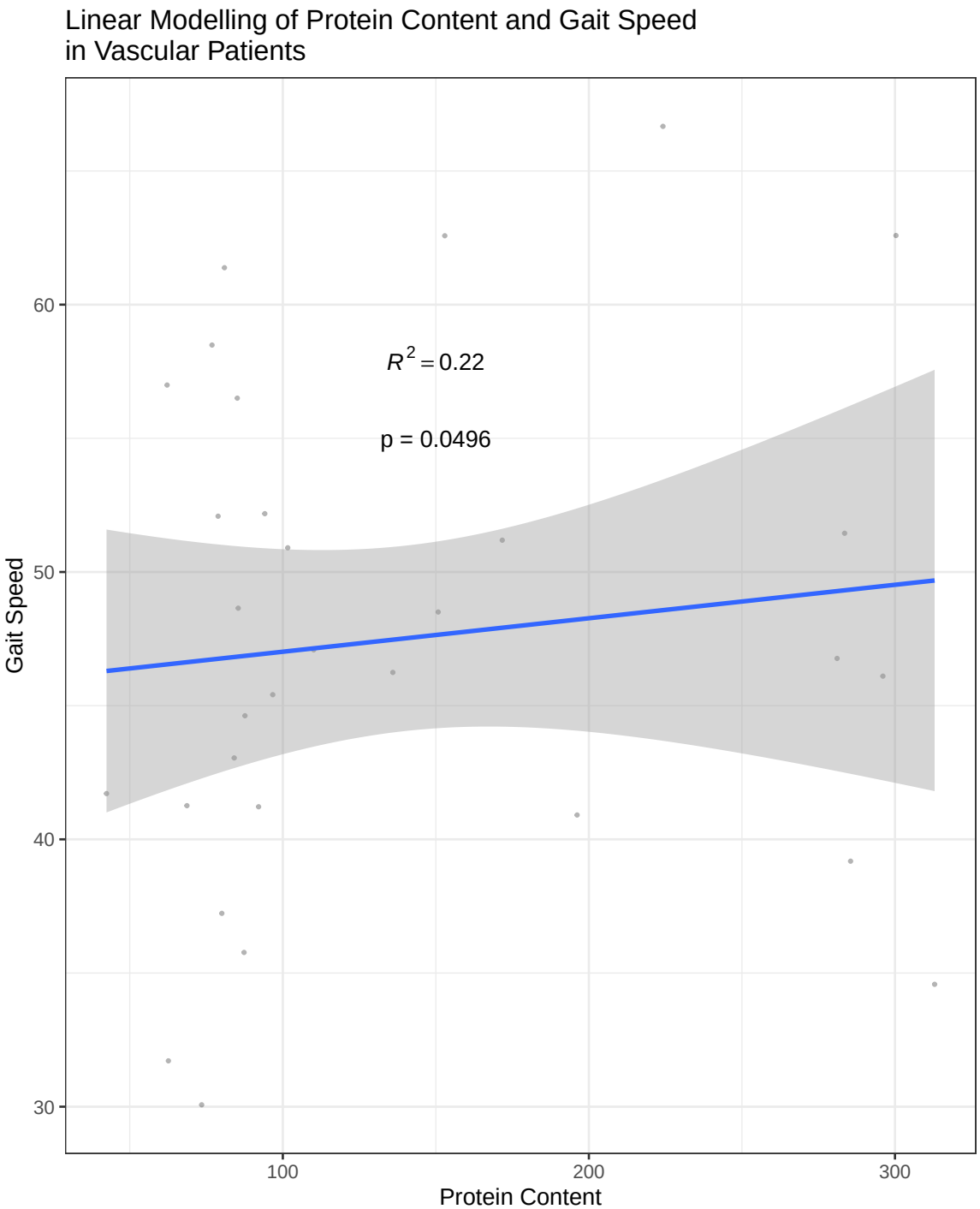



Figure 7.8: Linear Modelling of Protein Content and Gait Speed in Vascular Patients

### 7.3.6 Protein content distribution and clinical phenotypes

As mentioned in Chapter 1, Johns *et al* [66] investigated the distribution of skeletal muscle protein content according to 4 clinical phenotypes: 1. Weight loss >5% 1. Weight loss >10% 1. Low muscularity by CT-BCA 1. Low muscularity by CT-BCA and weight loss >2%

In their study, Johns *et al* recruited 32 upper GI cancer patients who underwent rectus abdominis biopsies, finding statistically significant differences in protein content between those who did and did not exhibit class I and class IV phenotypes. The present patient cohort was investigated to ascertain whether these clinical phenotypes would be applicable, whether differences in protein content would reach statistical significance, and which classes of phenotype would reach significance. Applying the clinical phenotypes to all groups revealed statistically significant differences in protein content in the class II phenotype only (132 v 96.3 microg/mg wet weight, $p = 0.0045$). Differences were seen in both the AAA (144 v 77.2 microg/mg wet weight, $p = 0.0004$) and UGIC (128 v 96.5 microg/mg wet weight, $p = 0.0289$) groups, however on closer inspection it was revealed that there were only 2 patients in the AAA group who fulfilled the class II phenotype. As such, it is unclear how much this result can be relied upon. These results are presented in tabular form in Table 7.10 and Table 7.11.

Table 7.10: Welch 2-sided t-test of Mean Skeletal Muscle Protein Content In All Patients

| Difference | Normal | Class II phenotype | Low CI | High CI | p-value |
|---|---|---|---|---|---|
| 35.2505 | 131.5053 | 96.2548 | 11.4651 | 59.0359 | 0.0045 |

Table 7.11: Welch 2-sided t-test of Mean Skeletal Muscle Protein Content In Patients Who Do And Do Not Meet Class II Phenotype

| Group | Difference | Normal | Class II phenotype | Low CI | High CI | p-value |
|---|---|---|---|---|---|---|
| Vascular | 67.1753 | 144.3865 | 77.2112 | 33.1097 | 101.2408 | 4e-04 |
| Cancer | 31.1728 | 127.7026 | 96.5297 | 3.3672 | 58.9785 | 0.0289 |

## 7.4 Discussion of Relationship Between Skeletal Muscle Protein Content and CT Variables

The plots presented above, together with the tables detailing the statistical tests performed to demonstrate differences in muscle protein content by CT-derived cut-points, show that there is not a clear relationship between CT body composition measures of skeletal muscle and the protein content of that muscle. Accordingly, attempting to draw conclusions about the content of skeletal muscle from CT-derived body composition variables is fraught with difficulty and the potential to cause or report erroneous results. This is of relevance when intervention trials use CT-BCA as outcome measures assuming that changes in CT-BCA can act as a surrogate for changes in muscle protein content.

Additionally, the relationship between the protein content of skeletal muscle and objective physical performance of patients similarly does not follow a linear trajectory. It is likely that the functioning of skeletal muscle in patients seen clinically is not solely reliant on the protein content of that muscle. Other factors including changes at the neuro-muscular junction; previous physical training; and even patient motivation or mood may have an effect on how well the muscle functions, and how well that function can be translated into measurable outcomes.

Recently, increasing interest in the relationship of CT variables to measurable patient outcomes has led to an inference that certain variables relate to particular biochemical and biophysiological pathways and mechanisms and components. The current analysis lend credence to the suggestion that these relationships may be more complex and more difficult to interpret than previously considered.

# Chapter 8

# Relationship Between CT-BCA Variables and Muscle Fibre Cross-sectional Area (CSA)

As discussed in Chapter 1, work has previously been published investigating the differences in muscle fibre cross-sectional area (CSA) between patients with cachexia, and those without [66]. This work was confined to 41 cancer patients, and CSA distribution in healthy controls is unknown. Additionally, the work done by Johns *et al* utilised CT-BCA to classify patients into normal or low muscularity subgroups rather than investigating whether there is a relationship between CSA and CT-BCA.

## 8.1 Hypothesis and research questions

### 8.1.1 Hypothesis

Muscle CSA distribution differs between patient types; will separate into distinct subgroups when CT-BCA cut-points are applied; has a strong relationship with CT-BCA variables; and has a strong relationship with muscle protein content.

### 8.1.2 Research Questions

- What is the distribution of muscle CSA in each patient type?
- Can a separation be seen in CSA distribution when CT-BCA is applied?
- What is the relationship between CSA and CT-BCA?
- What is the relationship between CSA and protein content?

## 8.2 Methods for Assessing the relationship between CT and CSA

Between 2015 and 2017, patients from upper GI cancer (UGIC, n=56), vascular (AAA, n=30), and live kidney donors (LDN, n=24) were recruited. Skeletal muscle biopsies from the rectus abdominis muscle were collected, processed, stored, and analysed as described in Chapter 3.

Statistics and plotting were performed in R [168]. Analyses included curve fit using locally estimated scatterplot

smoothing (LOESS) modelling; weighted mean calculation; and t-tests and linear regression on the weighted mean. Linear regression is reported using Adjusted $R^2$.

## 8.3 Results of Skeletal Muscle Fibre CSA Analysis

The distribution of muscle fibre CSA in each patient type is presented in Figure 8.2for FTIIa (fast) fibres, and in Figure 8.3 for Non-FTIIa (slow) fibres. As shown, every patient has data points for each fibre size, with associated percentage composition of the light field as a percentage. Accordingly, the percentage composition and fibre size were combined using a weighted mean (WM-CSA) analysis to give individual patients a single value for muscle fibre size thus allowing comparisons between patients. Individual patient CSA examples are shown in Figure 8.1.

Weighted mean CSA is presented in Table 8.1 for FTIIa (fast) fibres and in Table 8.2 for Non-FTIIa (slow) fibres. The reader will note that the figures for each match exactly - perhaps in part related to the numbers of patients in each subset. To ensure there was indeed a difference between fibre type CSA, WM-CSA was analysed across all patients purely by sex, as shown in Table 8.3. This reveals that the WM-CSA is indeed different between fibre types, and thus the matching numbers seen in the tables referred to above may be related to patient numbers; to the loss of data granularity incurred in calculating a weighted mean; or to a combination of both.

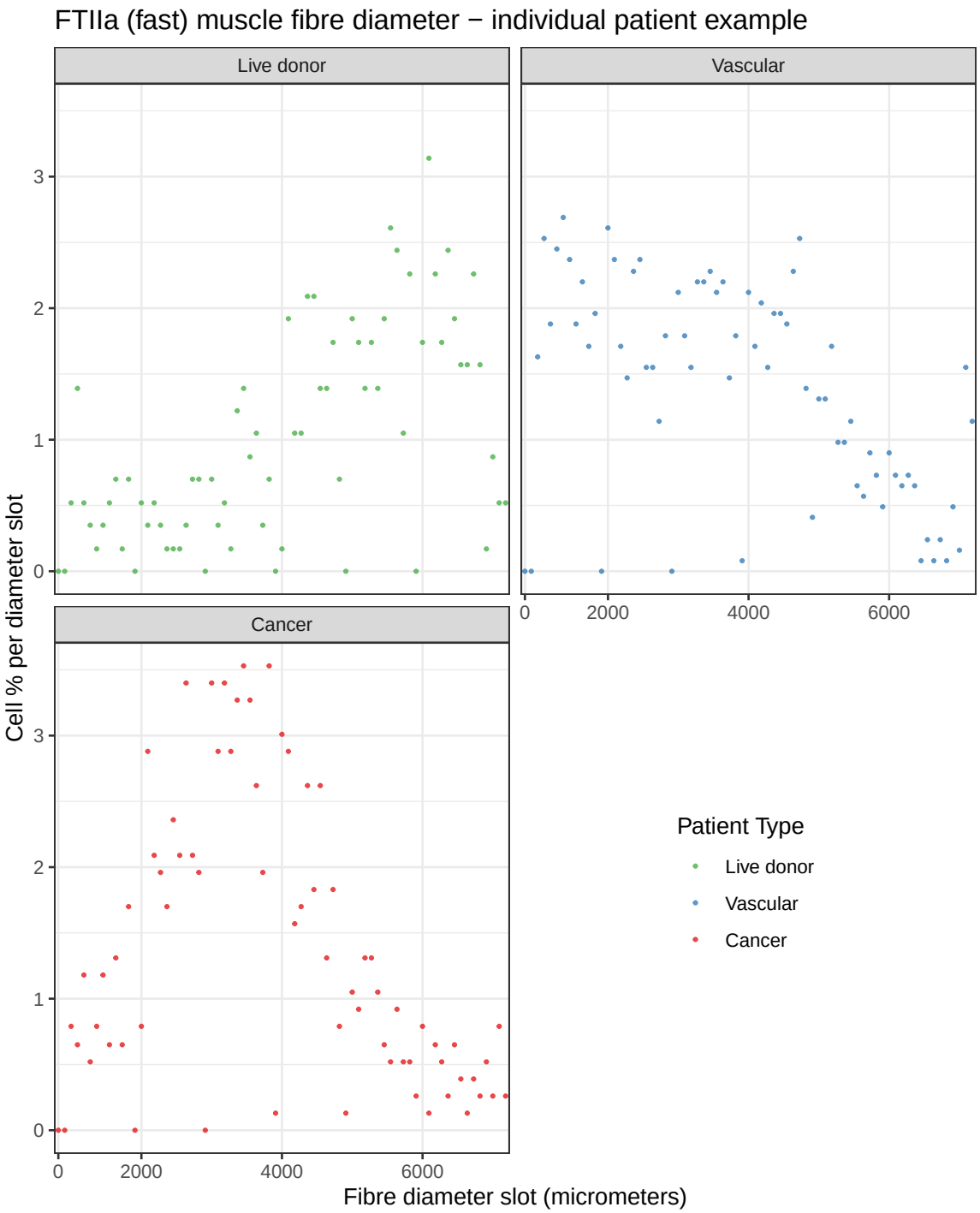



Figure 8.1: Individual Patient FTIIa (fast) fibre CSA Distribution

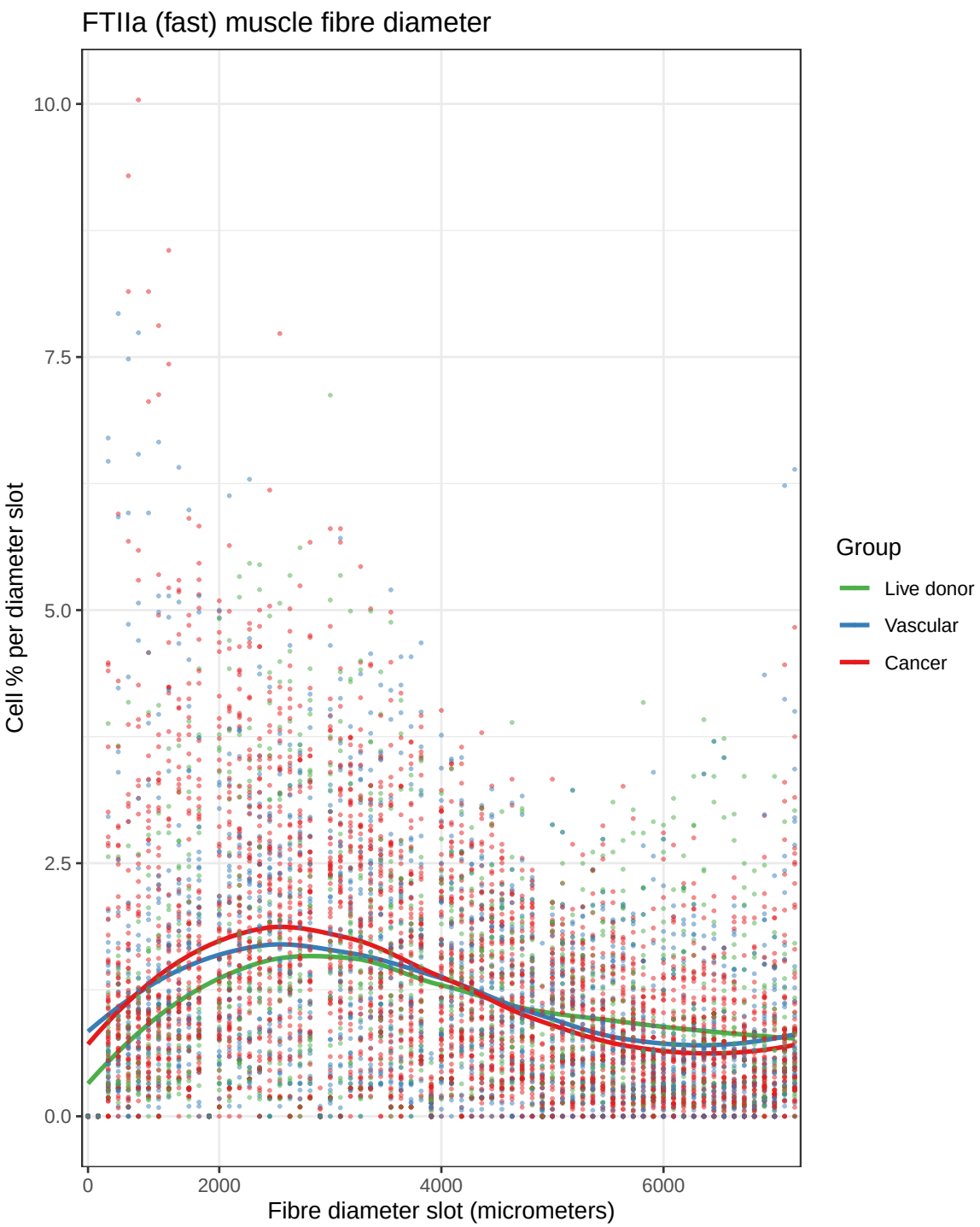



Figure 8.2: CSA Distribution in All Patients for FTIIa (fast) fibres

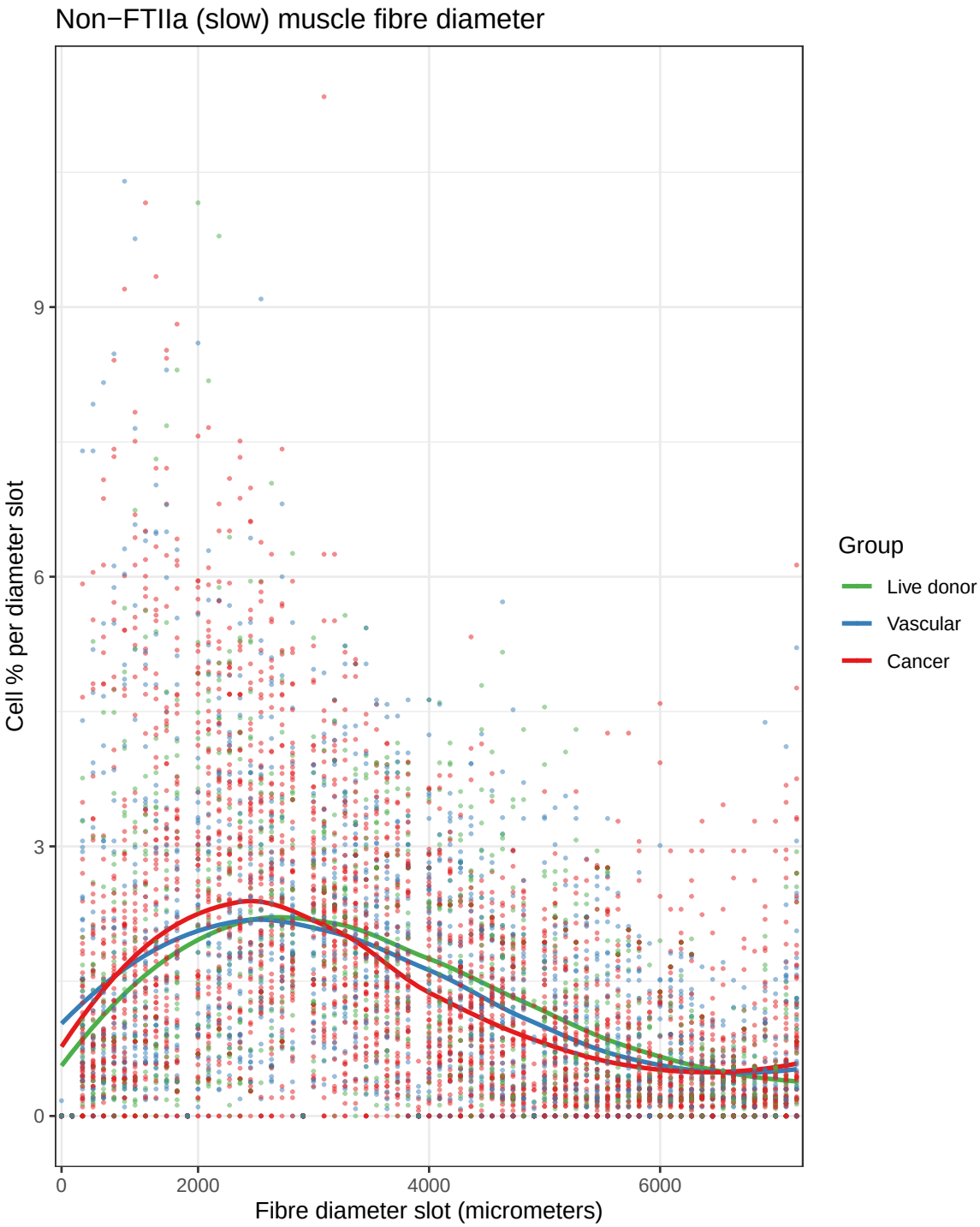



Figure 8.3: CSA Distribution in All Patients for Non-FTIIa (slow) fibres

Table 8.1: Weighted Mean FTIIa (fast) Muscle Fibre CSA by Patient Type, Sex, and BMI

| Patient Type | Sex | BMI | Min | Max | Mean (sd) | n |
|---|---|---|---|---|---|---|
| **Live Donor** | | | | | | |
| | Male | Normal | 2730.24 | 3812.99 | 3155.77 (464.52) | 4 |
| | Male | High | 4345.16 | 5484.92 | 4805.92 (419.47) | 8 |
| | Female | Normal | 2392.60 | 2392.60 | 2392.6 (NA) | 1 |
| | Female | High | 2248.12 | 4577.25 | 3520.45 (643.95) | 11 |
| **Vascular** | | | | | | |
| | Male | Normal | 2976.55 | 5110.07 | 3652.46 (656.94) | 8 |
| | Male | High | 1673.39 | 5127.23 | 3400.81 (994.29) | 14 |
| | Female | Normal | 2977.47 | 3962.92 | 3506.32 (438.17) | 4 |
| | Female | High | 1608.34 | 4423.58 | 2968.95 (1173.15) | 4 |
| **Cancer** | | | | | | |
| | Male | Normal | 2302.23 | 4752.08 | 3433.2 (771.83) | 15 |
| | Male | High | 1671.25 | 4829.34 | 3554.84 (756.36) | 25 |
| | Female | Normal | 1954.56 | 4021.06 | 2863.09 (758.11) | 8 |
| | Female | High | 1813.24 | 4577.25 | 3468.48 (934.46) | 8 |

Table 8.2: Weighted Mean Non-FTIIa (slow) Muscle Fibre CSA by Patient Type, Sex, and BMI

| Patient Type | Sex | BMI | Min | Max | Mean (sd) | n |
|---|---|---|---|---|---|---|
| **Live Donor** | | | | | | |
| | Male | Normal | 2730.24 | 3812.99 | 3155.77 (464.52) | 4 |
| | Male | High | 4345.16 | 5484.92 | 4805.92 (419.47) | 8 |
| | Female | Normal | 2392.60 | 2392.60 | 2392.6 (NA) | 1 |
| | Female | High | 2248.12 | 4577.25 | 3520.45 (643.95) | 11 |
| **Vascular** | | | | | | |
| | Male | Normal | 2976.55 | 5110.07 | 3652.46 (656.94) | 8 |
| | Male | High | 1673.39 | 5127.23 | 3400.81 (994.29) | 14 |
| | Female | Normal | 2977.47 | 3962.92 | 3506.32 (438.17) | 4 |
| | Female | High | 1608.34 | 4423.58 | 2968.95 (1173.15) | 4 |
| **Cancer** | | | | | | |
| | Male | Normal | 2302.23 | 4752.08 | 3433.2 (771.83) | 15 |
| | Male | High | 1671.25 | 4829.34 | 3554.84 (756.36) | 25 |
| | Female | Normal | 1954.56 | 4021.06 | 2863.09 (758.11) | 8 |
| | Female | High | 1813.24 | 4577.25 | 3468.48 (934.46) | 8 |

Table 8.3: Weighted Mean CSA by Sex and Fibre Type

| Sex | Fibre Type | Min | Max | Mean (sd) |
|---|---|---|---|---|
| **Male** | | | | |
| | Non-FTIIa (slow) | 1632.51 | 5684.04 | 3198.2 (880.11) |
| | FTIIa (fast) | 1671.25 | 5484.92 | 3625.28 (854.03) |
| **Female** | | | | |
| | Non-FTIIa (slow) | 1829.62 | 4258.24 | 3139.25 (738.08) |
| | FTIIa (fast) | 1608.34 | 4577.25 | 3268.64 (804.85) |

### 8.3.1 Relationship Between CSA and CT-BCA

As described by Johns *et al* [66], differences have been noted in muscle CSA between patients with low muscularity as defined by CT and those with normal muscularity. The present study aimed to utilise the cohort described above to more closely define this relationship.

#### 8.3.1.1 SMI and SMI Cut-points

When investigating CSA and SMI, it seems reasonable to ascertain whether there is a relationship between the two. Linear regression performed between SMI and CSA did not, however, reveal any statistically significant relationship. For FTIIa (fast) fibres, using a linear model, $R^2$ = 0.01 with p = 0.144. For Non-FTIIa (slow) fibres using the same linear model, $R^2$ = -0.07 with p = 0.644. These results are plotted in Figure 8.4 for FTIIa (fast) fibres, and Figure 8.5 for Non-FTIIa (slow)fibres.

Considering that subgroup analysis may reveal a difference between groups for weighted mean CSA, attention was turned to the Martin *et al* [18] subgroups within each cohort. The t-tests between *normal* and *low* SMI groups for CSA are noted in Table 8.4 for FTIIa (fast) and in Table 8.5 for Non-FTIIa (slow) fibres.

Linear regression modelling in these subgroups revealed no statistically significant relationship between SMI and WM-CSA.

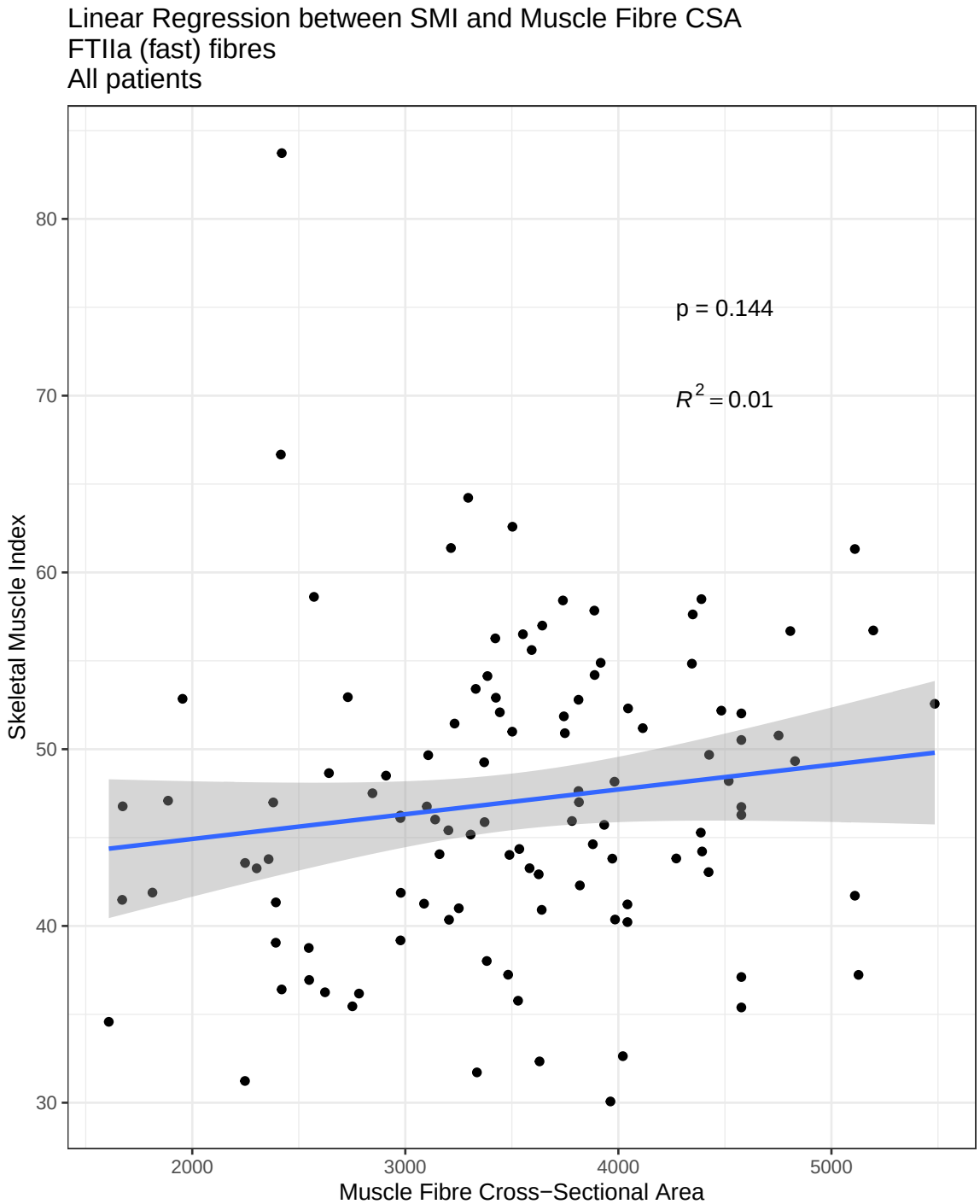



Figure 8.4: Relationship Between WM-CSA and SMI in FTIIa (fast) fibres

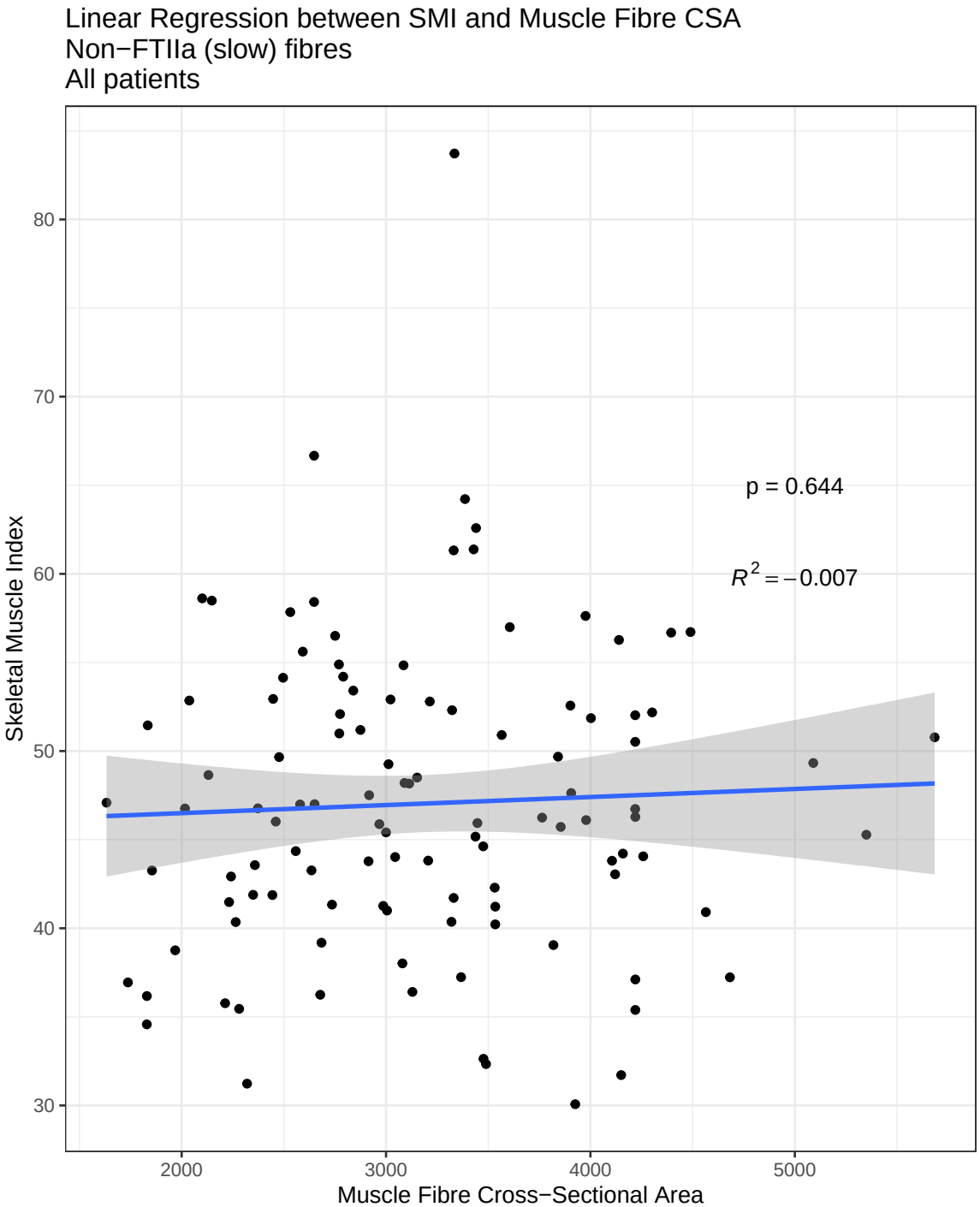



Figure 8.5: Relationship Between WM-CSA and SMI in Non-FTIIa (slow) fibres

Table 8.4: Welch 2-sided t-test of FTIIa (fast) Muscle Fibre CSA by Sex and Patient Group Between Normal and Low SMI

| Patient Type | Sex | Difference | Normal SMI | Low SMI | Confidence Interval (Low) | Confidence Interval (High) | p value |
|---|---|---|---|---|---|---|---|
| **Live donor** | | | | | | | |
| | Male | -223.01 | 4181.53 | 4404.54 | -1763.87 | 1317.85 | 0.73 |
| | Female | -175.41 | 3353.37 | 3528.78 | -1048.20 | 697.38 | 0.66 |
| **Vascular** | | | | | | | |
| | Male | -77.64 | 3446.44 | 3524.09 | -809.21 | 653.92 | 0.83 |
| | Female | 532.89 | 3504.08 | 2971.19 | -1026.63 | 2092.41 | 0.43 |
| **Cancer** | | | | | | | |
| | Male | -55.71 | 3482.76 | 3538.47 | -552.06 | 440.63 | 0.82 |
| | Female | 338.48 | 3313.87 | 2975.39 | -645.22 | 1322.17 | 0.47 |

Table 8.5: Welch 2-sided t-test of Non-FTIIa (slow) Muscle Fibre CSA by Sex and Patient Group Between Normal and Low SMI

| Patient Type | Sex | Difference | Normal SMI | Low SMI | Confidence Interval (Low) | Confidence Interval (High) | p value |
|---|---|---|---|---|---|---|---|
| **Live donor** | | | | | | | |
| | Male | -326.71 | 3369.52 | 3696.23 | -1598.72 | 945.29 | 0.56 |
| | Female | 58.51 | 3175.25 | 3116.74 | -849.31 | 966.34 | 0.89 |
| **Vascular** | | | | | | | |
| | Male | -74.16 | 3115.06 | 3189.22 | -771.44 | 623.12 | 0.83 |
| | Female | 56.98 | 3204.53 | 3147.55 | -1658.21 | 1772.17 | 0.94 |
| **Cancer** | | | | | | | |
| | Male | -225.98 | 3028.42 | 3254.40 | -826.26 | 374.30 | 0.45 |
| | Female | 43.98 | 3131.38 | 3087.40 | -785.10 | 873.05 | 0.91 |

#### 8.3.1.2 SMD and SMD Cut-points

Whilst a relationship between CSA and SMI could be possible, it seems more likely that there would be a relationship between CSA and SMD. This potential relationship could be explained by an increase in radio-density being affected either by an increase in intra-cellular radio-dense material in large muscle fibres, or by an increased proportion of extra-cellular or cell wall material in small muscle fibres.

Linear regression was performed for each fibre type across the entire cohort, and revealed $R^2$ = -0.009, with p = 0.965 for FTIIa (fast) fibres and $R^2$ = 0.003 with p = 0.253 for Non-FTIIa (slow) fibres.

Plots showing this graphically are seen in Figure 8.6 for FTIIa (fast) and in Figure 8.7 for Non-FTIIa (slow) fibres.

Subdividing the cohort and groups by SMD cut-points allowed t-testing between *normal* and *low* SMD as shown in Table 8.6 for FTIIa (fast) and Table 8.7 for Non-FTIIa (slow) fibres. There were too few LDN patients in the *normal* BMI group in each of the *SMD* subdivisions to perform a t-test. In the *High* BMI LDN subgroup, the differences between *normal* and *low* SMD did not reach statistical significance.

Overall, these tests do not reveal any significant difference between patient type WM-CSA values when divided by CT-BCA cut-points.

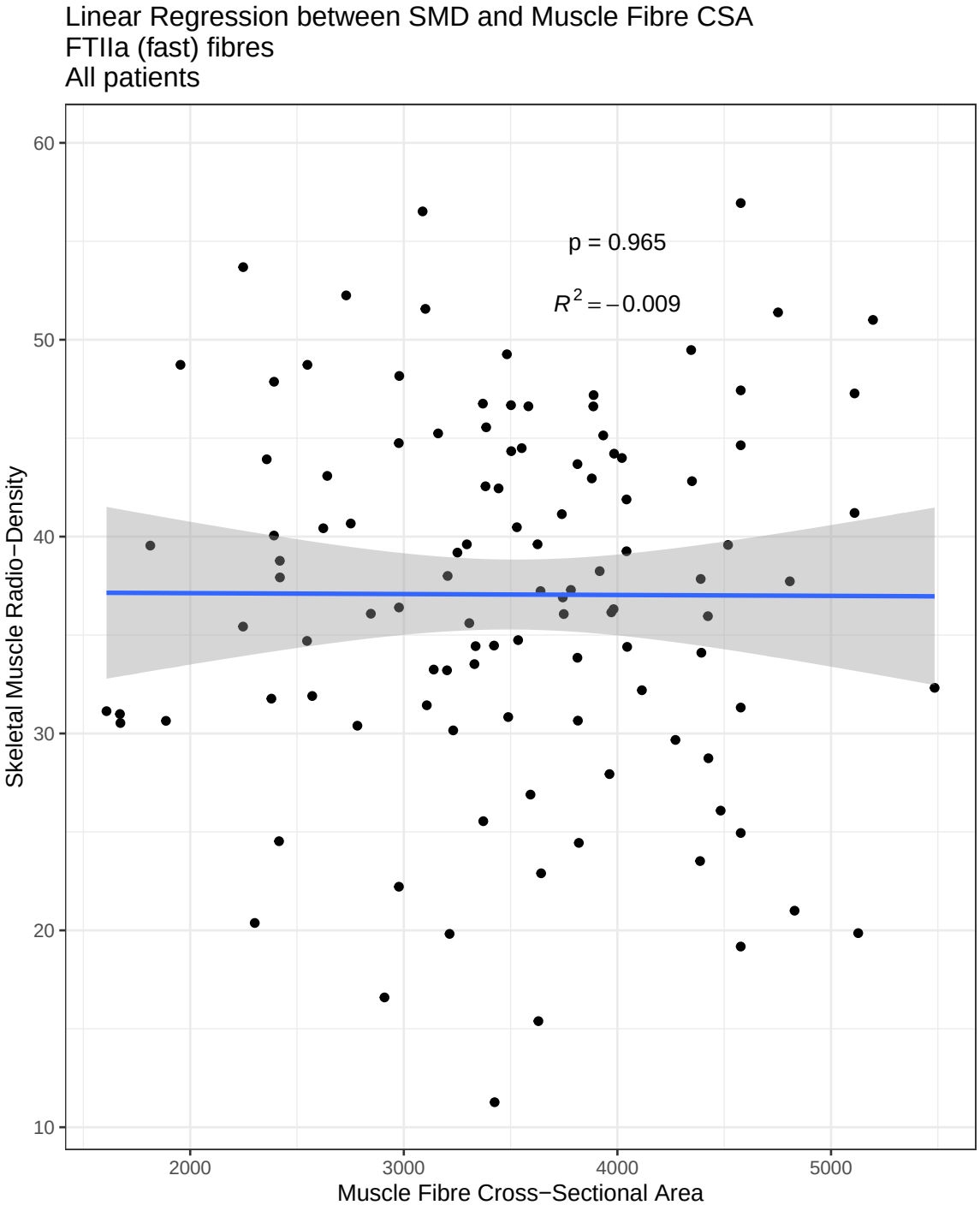



Figure 8.6: Relationship Between WM-CSA and SMI in FTIIa (fast) fibres

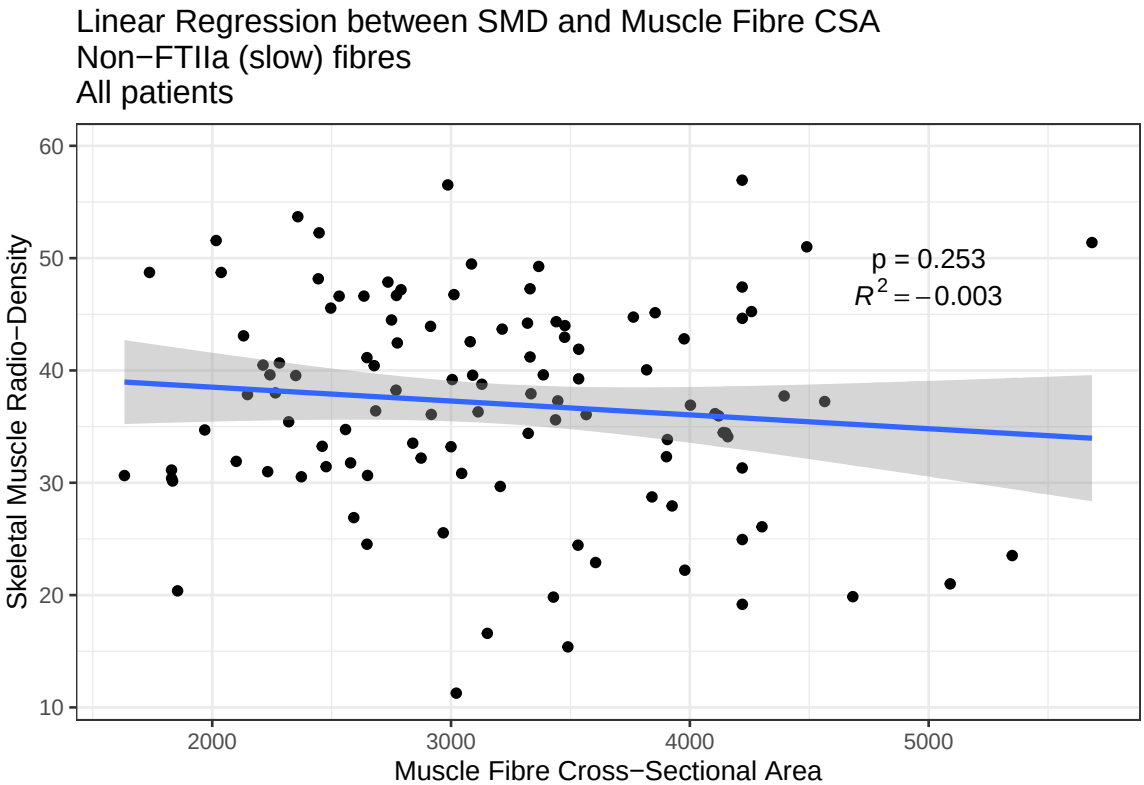



Figure 8.7: Relationship Between WM-CSA and SMD in Non-FTIIa (slow) fibres

Table 8.6: Welch 2-sided t-test of FTIIa (fast) Muscle Fibre CSA by Patient Type and by BMI Between Normal and Low SMD

| Patient Type | Patient BMI | Difference | Normal SMD | Low SMD | Confidence Interval (Low) | Confidence Interval (High) | p value |
|---|---|---|---|---|---|---|---|
| **Cancer** | | | | | | | |
| | Normal | 142.60 | 3675.05 | 3532.45 | -678.31 | 963.51 | 0.69 |
| | High | 593.45 | 3700.48 | 3107.03 | -330.68 | 1517.59 | 0.19 |
| **Vascular** | | | | | | | |
| | Normal | 286.35 | 3409.20 | 3122.85 | -515.92 | 1088.61 | 0.45 |
| | High | -253.37 | 3418.74 | 3672.10 | -838.61 | 331.88 | 0.38 |

Table 8.7: Welch 2-sided t-test of Non-FTIIa (slow) Muscle Fibre CSA by Sex and by Patient Group Between Normal and Low SMD

| Patient Type | Patient BMI | Difference | Normal SMD | Low SMD | Confidence Interval (Low) | Confidence Interval (High) | p value |
|---|---|---|---|---|---|---|---|
| **Cancer** | | | | | | | |
| | Normal | -336.95 | 3180.41 | 3517.36 | -1293.18 | 619.27 | 0.43 |
| | High | 33.95 | 3062.47 | 3028.52 | -914.30 | 982.20 | 0.94 |
| **Vascular** | | | | | | | |
| | Normal | 339.86 | 3108.12 | 2768.26 | -634.00 | 1313.72 | 0.46 |
| | High | -157.34 | 3216.23 | 3373.57 | -765.22 | 450.54 | 0.60 |

### 8.3.2 Protein Content and CSA

Intellectually, a linear relationship between CSA and muscle protein content could be expected. This was tested as shown in Figure 8.8 for FTIIa (fast) fibres, and Figure 8.9 for Non-FTIIa (slow) fibres. This suggests a very weak, though statistically significant relationship between protein content and WM-CSA in fast fibres.

To further investigate this, an assessment of this same relationship was performed for a series of CT-BCA-derived sub-groups.

There were no significant relationships noted between WM-CSA values in *normal* or *low* SMI or SMD groups for Non-FTIIa (slow) fibres.

For FTIIa (fast) fibres, there were statistically significant relationships noted, as presented in Table 8.8 for SMI, and in Table 8.9 for SMD. These show a statistically significant relationship only in the AAA cohort, and specifically only in those with *low* SMI or *low* SMD.

These relationships are *weak* but reach statistical significance.

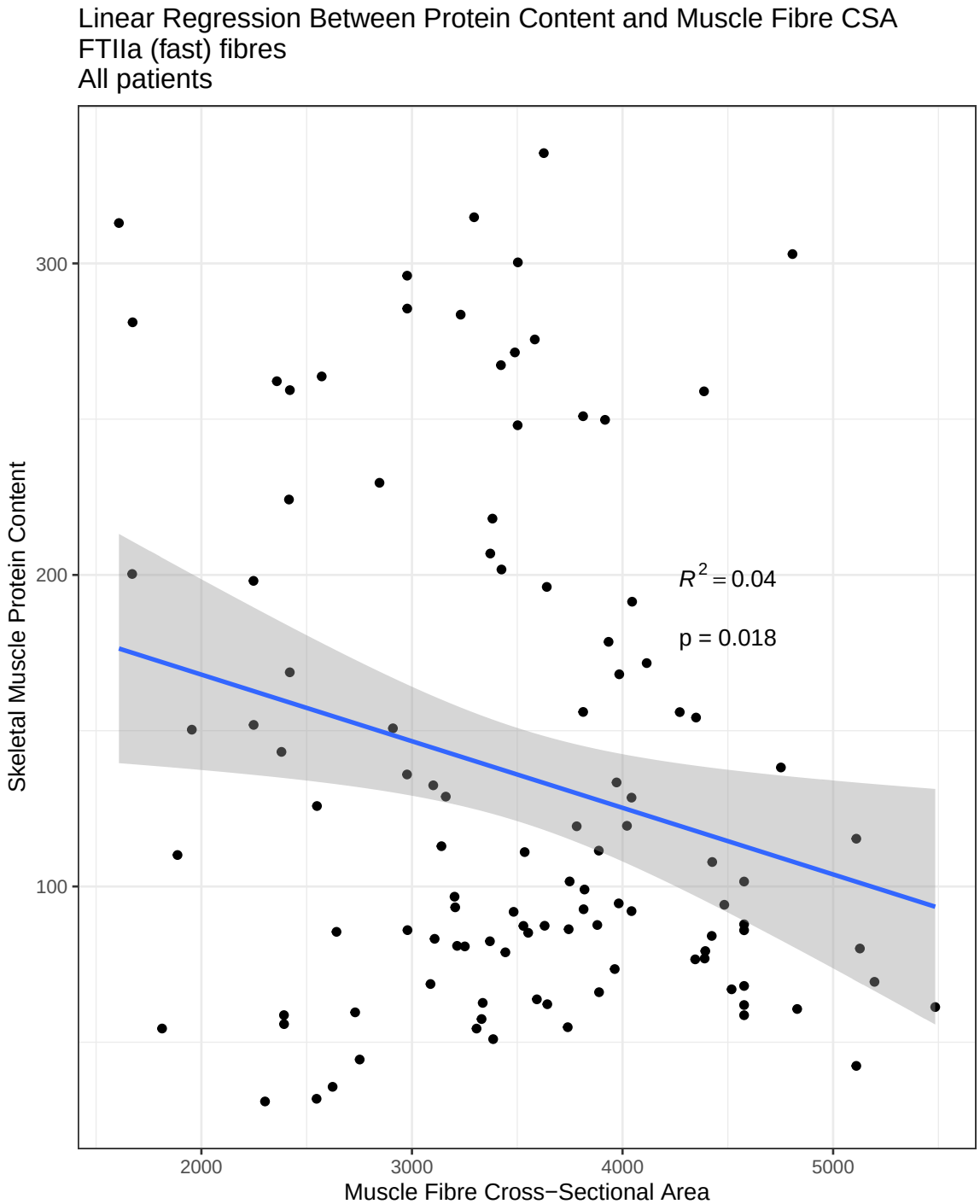



Figure 8.8: Relationship Between WM-CSA and Protein Content in FTIIa (fast) fibres

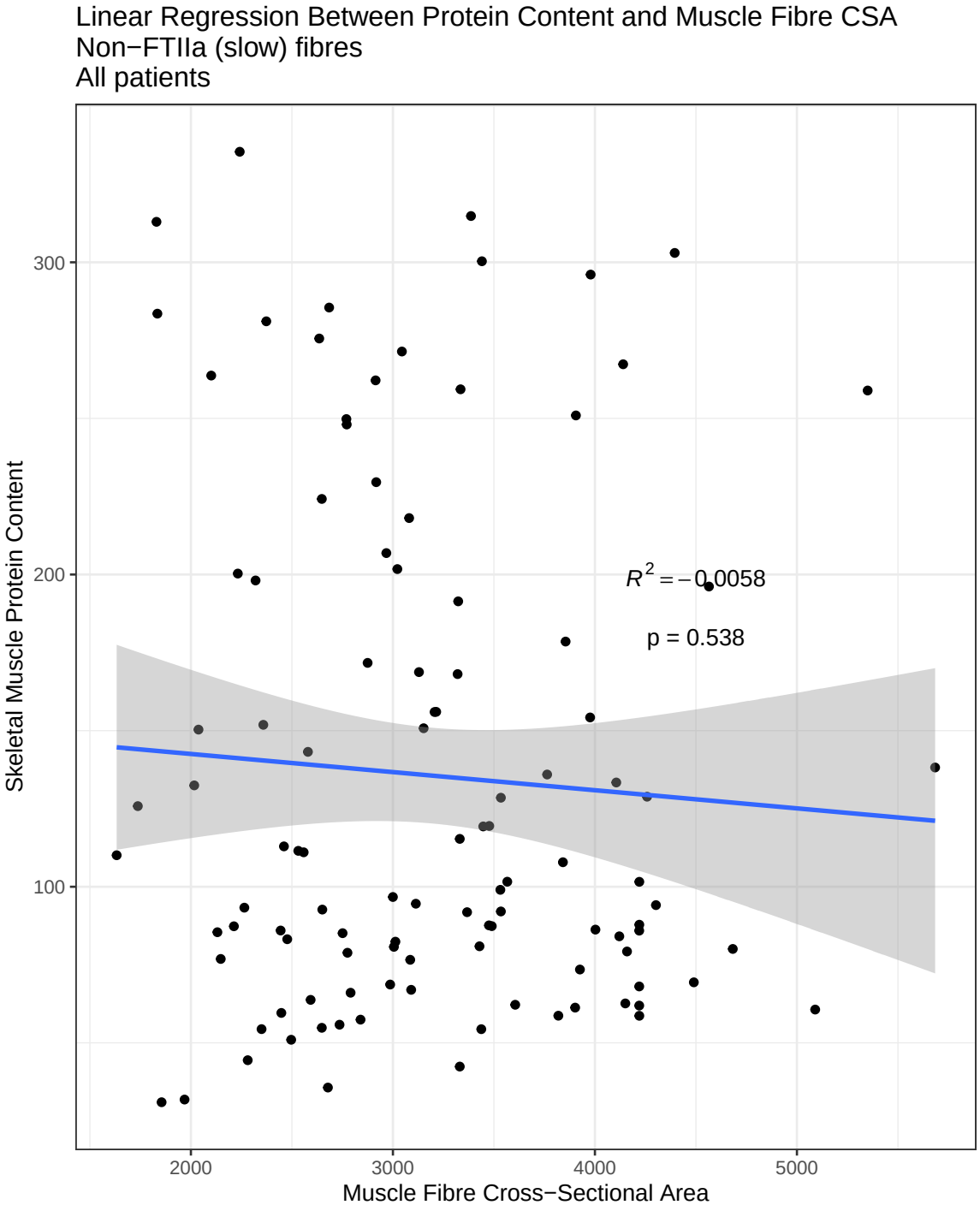



Figure 8.9: Relationship Between WM-CSA and Protein Content in Non-FTIIa (slow) fibres

Table 8.8: Linear Regression between FTIIa (fast) Muscle Fibre CSA and Protein Content in All Patients by SMI

| Patient Type | SMI | R-Squared (adjusted) | p value |
|---|---|---|---|
| **Live donor** | | | |
| | Normal SMI | -0.06 | 0.68 |
| | Low SMI | -0.11 | 0.65 |
| **Vascular** | | | |
| | Normal SMI | 0.04 | 0.24 |
| | Low SMI | 0.33 | 0.01 |
| **Cancer** | | | |
| | Normal SMI | -0.02 | 0.56 |
| | Low SMI | -0.01 | 0.41 |

Table 8.9: Linear Regression between Non-FTIIa (slow) Muscle Fibre CSA and Protein Content in All Patients by SMD

| Patient Type | SMD | R-Squared (adjusted) | p value |
|---|---|---|---|
| **Live donor** | | | |
| | Normal SMD | -0.03 | 0.5 |
| | Low SMD | - | - |
| **Vascular** | | | |
| | Normal SMD | -0.02 | 0.41 |
| | Low SMD | 0.29 | 0.01 |
| **Cancer** | | | |
| | Normal SMD | -0.01 | 0.37 |
| | Low SMD | -0.03 | 0.7 |

### 8.3.3 Gait Speed and CSA

A consideration of objective physical functioning and muscle CSA is presented in Figure 8.10 for FTIIa (fast) fibres, and Figure 8.11 for Non-FTIIa (slow) fibres. Linear regression subgroup analysis was performed to investigate the relationship between WM-CSA and gait speed in Non-FTIIa (slow) muscle fibres for CT-BCA subgroups (both SMI and SMD). This did not reveal any statistically significant relationships.

Similar subgroup analysis was performed for FTIIa (fast) fibres. In the LDN group, we were unable to demonstrate a relationship due to small numbers in each subgroup. Results from the UGIC and AAA groups are presented in Table 8.10 and in Table 8.11 for SMD and SMI respectively.

These tables suggest that there is in fact a relationship between WM-CSA and gait speed when sub-grouped by CT-BCA, but that it is confined to subsets of AAA patients. Of note, within the AAA, normal BMI, low SMI subset there are only 3 patients. As such, it is unclear whether chance may explain this relationship, and indeed whether this relationship will continue in the face of a greater sample size.

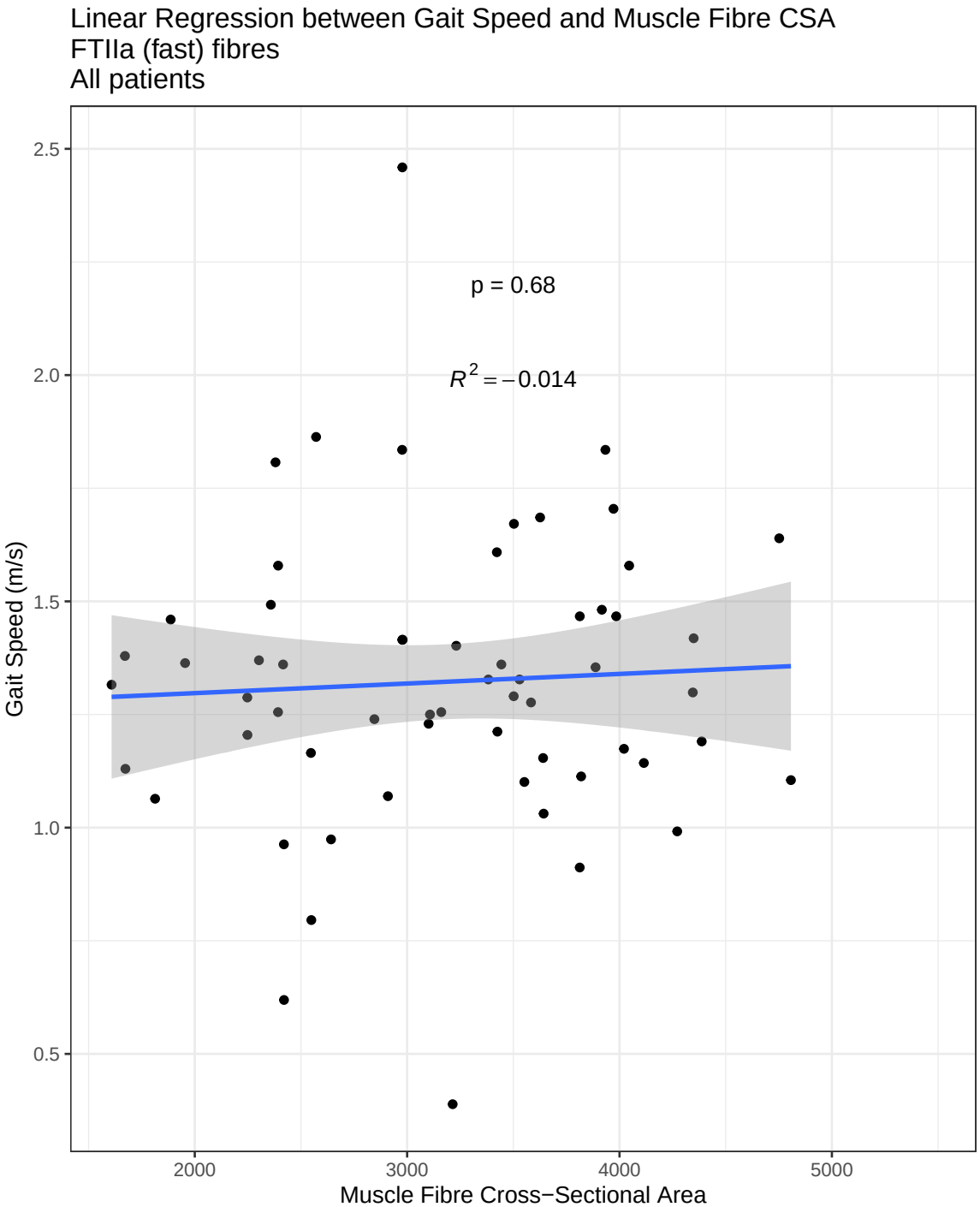



Figure 8.10: Relationship Between WM-CSA and Gait Speed in FTIIa (fast) fibres

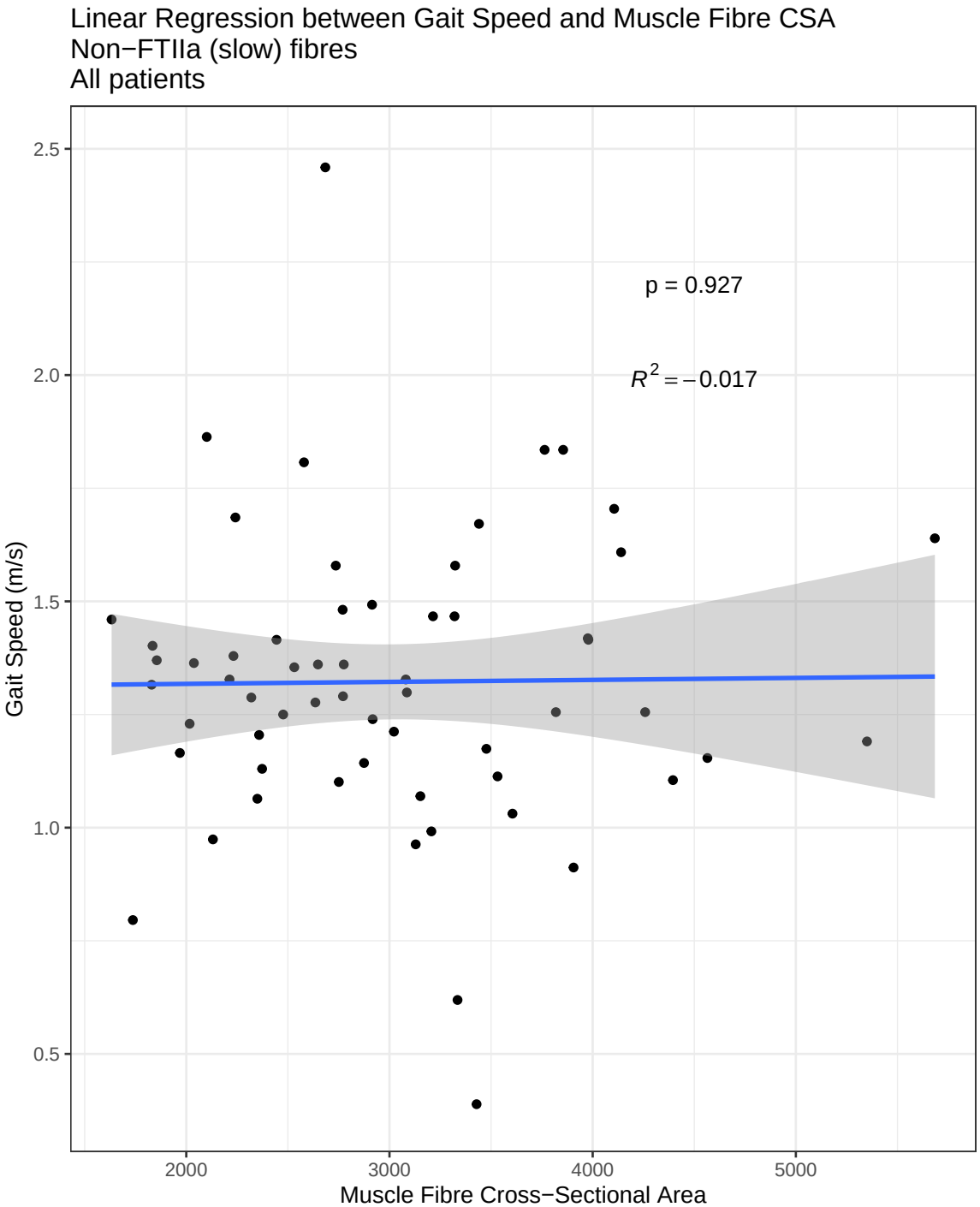



Figure 8.11: Relationship Between WM-CSA and Gait Speed in Non-FTIIa (slow) fibres

Table 8.10: Linear Regression between FTIIa (fast) Muscle Fibre CSA and Gait Speed, by SMD

| Patient Type | BMI | SMD | R-Squared (adjusted) | p value |
|---|---|---|---|---|
| **Vascular** | | | | |
| | Normal | Normal SMD | 0.94 | 0.11 |
| | Normal | Low SMD | 1 | 0.02 |
| | High | Normal SMD | - | - |
| | High | Low SMD | 0 | 0.34 |
| **Cancer** | | | | |
| | Normal | Normal SMD | -0.08 | 0.49 |
| | Normal | Low SMD | 0.88 | 0.01 |
| | High | Normal SMD | 0.61 | 0.01 |
| | High | Low SMD | 0.4 | 0.06 |

Table 8.11: Linear Regression between FTIIa (fast) Muscle Fibre CSA and Gait Speed, by SMI

| Group | BMI | SMI | R-Squared (adjusted) | p value |
|---|---|---|---|---|
| **Vascular** | | | | |
| | Normal | Normal SMI | 0.94 | 0.11 |
| | Normal | Low SMI | 1 | 0.02 |
| | High | Normal SMI | -0.33 | 0.99 |
| | High | Low SMI | -0.16 | 0.69 |
| **Cancer** | | | | |
| | Normal | Normal SMI | -0.2 | 0.97 |
| | Normal | Low SMI | -0.33 | 0.98 |
| | High | Normal SMI | -0.16 | 0.71 |
| | High | Low SMI | -0.12 | 0.97 |

### 8.3.4 Multivariable Regression in CSA

Having considered the relationships between individual variables and WM-CSA for both FTIIa (fast) and Non-FTIIa (slow) fibres, a multivariable analysis was then conducted to investigate and model potential predictors of WM-CSA in this context. As discussed in Chapter 3, initial additive modelling was conducted, and checked by stepwise removal.

When the FTIIa (fast) fibre types were considered, the variables identified for further interest were sex, age, and protein content. This analysis revealed the model shown in Table 8.12. Closer inspection of this model, however, reveals that the Akaike information criterion indicates poor model fitting, and so the interpretability of this model is unclear. Additionally, it is to be noted that none of the identified variables were CT-BCA variables which suggests that CT-BCA variables are not the strongest predictors of WM-CSA in this population.

When Non-FTIIa (slow) fibre types were considered, there was only one variable identified for model construction - weight. Since a single-variable model cannot be considered as a multivariable analysis this was not included. It is interesting to note, however, that CT-BCA variables were once again not identified as predictor variables in this case.

Table 8.12: Multivariable Modelling of WM-CSA Predictors in FTIIa (fast) Fibres

| Dependent: WM-CSA | | unit | value | Coefficient (univariable) | Coefficient (multivariable) |
|---|---|---|---|---|---|
| Sex | Male | Mean (sd) | 3625.3 (854.0) | - | - |
| | Female | Mean (sd) | 3268.6 (804.9) | -356.63 (-694.33 to -18.94, p=0.039) | -387.00 (-712.38 to -61.63, p=0.020) |
| Age | [29.0,90.0] | Mean (sd) | 3508.6 (851.3) | -17.66 (-30.95 to -4.37, p=0.010) | -14.47 (-27.50 to -1.44, p=0.030) |
| Protein Content | [31.0,335.4] | Mean (sd) | 3508.6 (851.3) | -2.40 (-4.37 to -0.42, p=0.018) | -2.27 (-4.20 to -0.34, p=0.022) |
| Number in dataframe = 226, Number in model = 109, Missing = 117, Log-likelihood = -881.35, AIC = 1772.7, R-squared = 0.14, Adjusted R-squared = 0.12 | | | | | |

### 8.3.5 Defining Normality

Utilising the muscle biopsy results obtained from the LDN population, an attempt to define *"normal"* WM-CSA was undertaken.

In line with previous work on *sarcopenia* by Baumgartner *et al* [7], the mean and standard deviation for the LDN population were obtained for WM-CSA. These are is shown in Figure 8.12 for FTIIa (fast) fibres, and Figure 8.13 for Non-FTIIa (slow) fibres. These show very few patients below the cut-point, suggesting that very few patients have a *low* WM-CSA. Additionally, the WM-CSA is a crude measure of rather granular data, and as such it may be that it is not optimum for this particular task.

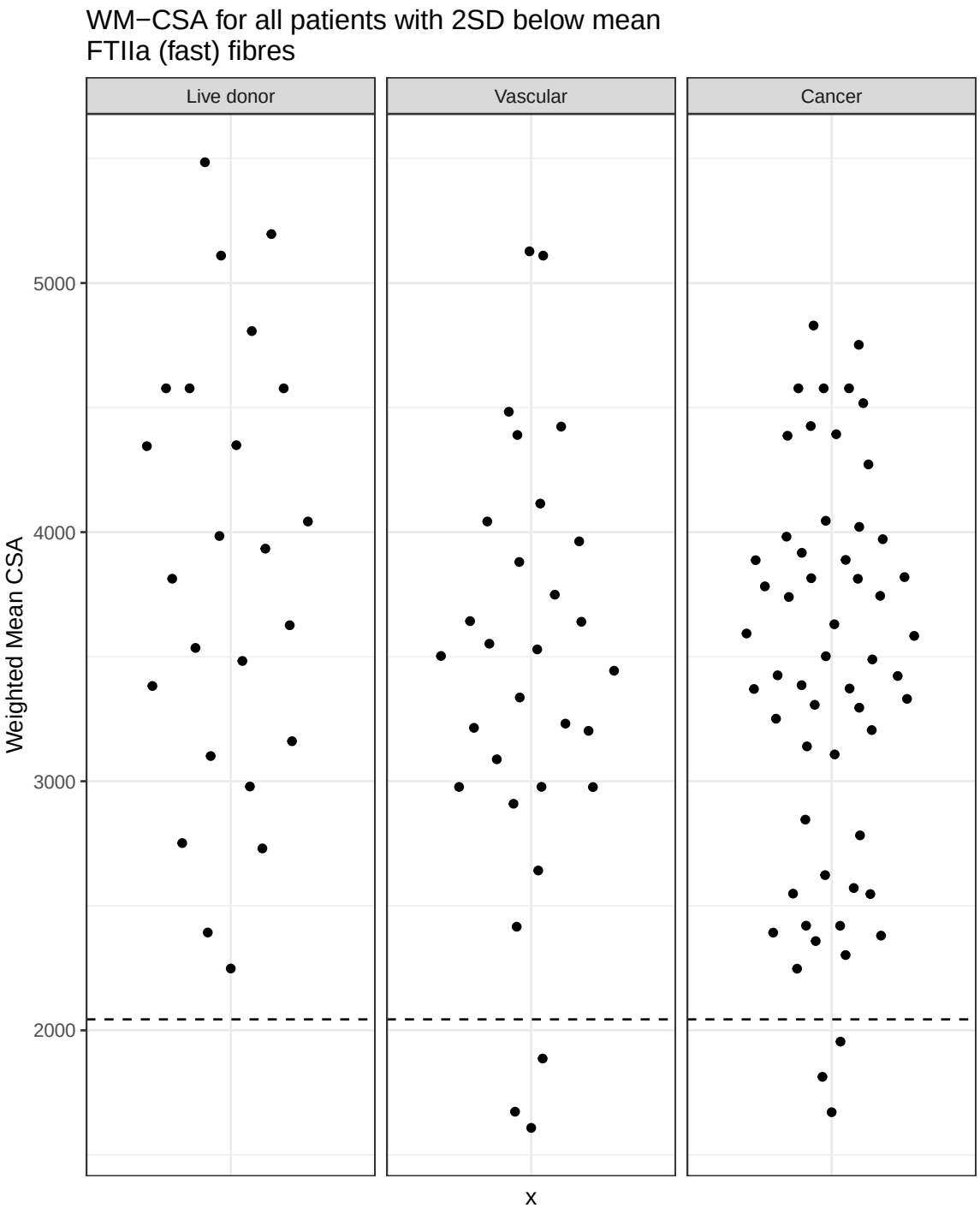



Figure 8.12: Weighted-Mean CSA with Normal Line (fast fibres)

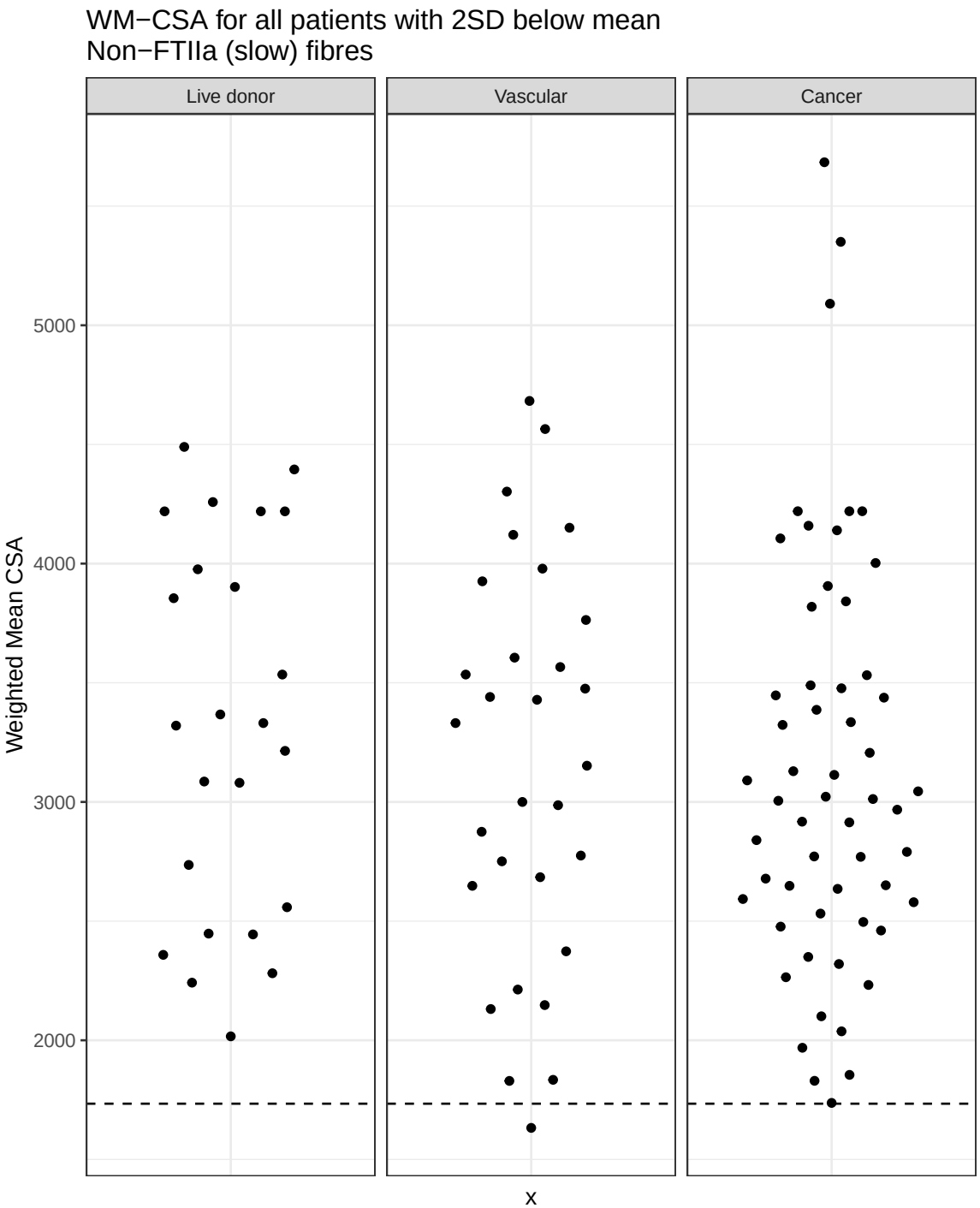



Figure 8.13: Weighted-Mean CSA with Normal Line (slow fibres)

# Chapter 9

# Relationship Between CT-BCA Variables and Inflammation

As described in Chapter 1, the effect of SI on cancer patient outcomes can be quite marked. Additionally, alterations in CT-BCA variables appear to relate to cancer patient outcomes and this has led to the development of cut-points below which patient outcomes appear to deteriorate. The relationship between SI and CT-BCA variables, however, is unclear; the effect of SI on CT-BCA and the potential clinical impact of this merits investigation, because SI is a significant factor in cachexia, and thus CT-BCA is influenced by the condition is purports to measure.

## 9.1 Hypothesis and Research Questions

### 9.1.1 Hypothesis

Systemic inflammation has minimal measurrable on CT-BCA variables.

### 9.1.2 Research Questions

- What differences are demonstrable between CT-BCA at differenct time-points prior to; during; and following severe SI?

- How do these differences, if any, relate to ongoing SI?

## 9.2 Methods for Investigating Relationship Between CT-BCA and Inflammation

Between August 2012 and October 2016, patients who underwent Ivor-Lewis oesophago-gastrectomy (ILOG), and who developed post-operative anastomotic leak, were identified retrospectively from a prospective local audit database. Routine preoperative staging CT scans closest to time of surgery were analysed with regards to body composition (*preop scans*). Those patients who developed symptoms or signs of sepsis (delirium, pain, pyrexia, tachycardia, hypotension, leucocytosis), or who on clinical grounds (drain fluid turbidity, increasing oxygen requirement) were suspected of suffering a breakdown of the oesophago-gastric intrathoracic anastomosis, underwent early postoperative CT scanning and these were analysed (*early scans*). Patients were excluded from analysis if an early postoperative CT scan was not available. Where available, any delayed

follow-up scans were also retrieved for analysis to assess the reversibility of changes noted on post-operative scans (*late scans* after sufficient time for resolution of SI). If the formal report from the radiologist indicated recurrent disease or an ongoing inflammatory process, these follow-up scans were excluded from subsequent analysis to exclude the confounding possibility of altered body composition because of tumour-derived metabolic derangement (cachexia) or ongoing SI. Following major surgery, patients are known to become significantly oedematous, leading to a well-recognised increase in body weight [175]. However, due to their critical illness state, patients are not routinely weighed during standard clinical care in the early postoperative period. For this reason, stratification of CT body composition results by patient BMI to identify sarcopenia was not performed, as the preoperative weight is unlikely to remain accurate in the postoperative phase, and furthermore, the influence of excess oedema on stratification calculations is unknown. Accordingly, the current study is focussed on tissue area measurements. Preoperative, early postoperative, and late follow-up CT scans were analysed as described in Chapter 3. Routine blood sampling was performed, including plasma C-reactive protein (CRP) levels measured by automated turbidimetry, and plasma albumin levels measured by dye-binding techniques. Variables were compared between the preoperative, postoperative, and follow-up groups using a Paired Sample t-test in R [168].

## 9.3 Results for Analysis of Relationship Between CT-BCA and Inflammation

During the study period, 18 patients with anastomotic leak who had undergone ILOG met criteria for inclusion. Demographic data are presented in Table 9.1. Postoperative scans were performed at median 9 days post-op (range 5-44), and follow-up scans at 430 days (126-1191). The CT variables shown in Table 9.1 are represented in Figure 9.1 for SMA, and in Figure 9.2 for SMD. Mean and standard deviation representations of this data are shown in Figure 9.3 for SMA and in Figure 9.4 for SMD.

These figures clearly demonstrate in graphical form the differences across time-points enumerated in Table 9.1. Between the preoperative, early, and late scans it can be seen that SMA first rises and then dips; and SMD dips and then rises. This combination of changes, in conjunction with a known elevation of inflammatory marker (CRP), suggests that these CT-BCA alterations are linked to the patients' ongoing and underlying inflammatory response.

Whilst visually appealing, a consideration of the figures will reveal that the error-bars, which are set at 1 standard

deviation from the mean, appear to overlap. It may be, therefore, that the visible differences represent a chance finding only. Accordingly, we proceeded to undertake a series of paired t-tests to ascertain whether there was a difference between the *preop*, *early*, and *late* time-points in order to determine firstly whether the dip or rise represented a true change, and secondly whether the recovery value (late) retained a significant difference from the initial value (preop).

Table 9.1: Demographic distribution of inflammatory patient data

| Dependent: Sex | | Male | Female | p |
|---|---|---|---|---|
| Age | Mean (SD) | 63.8 (9.0) | 62.7 (9.0) | 0.851 |
| Tumour type | ACC | 13 (81.2) | 3 (100.0) | 1.000 |
| | SCC | 3 (18.8) | | |
| Final_stage | 1 | 5 (31.2) | | 0.405 |
| | 3 | 6 (37.5) | 1 (33.3) | |
| | 4 | 5 (31.2) | 2 (66.7) | |
| SMA (Preop) | Mean (SD) | 154.4 (19.7) | 85.4 (5.3) | <0.001 |
| SMA (Early) | Mean (SD) | 162.9 (28.4) | 122.9 (8.0) | 0.032 |
| SMA (Late) | Mean (SD) | 140.0 (19.4) | 123.7 (19.2) | 0.290 |
| SMD (Preop) | Mean (SD) | 32.1 (8.3) | 36.6 (7.3) | 0.391 |
| SMD (Early) | Mean (SD) | 28.1 (8.0) | 27.7 (7.3) | 0.934 |
| SMD (Late) | Mean (SD) | 33.7 (5.8) | 30.4 (4.8) | 0.456 |
| CRP (Highest) | Mean (SD) | 232.6 (119.1) | 172.0 (104.5) | 0.429 |

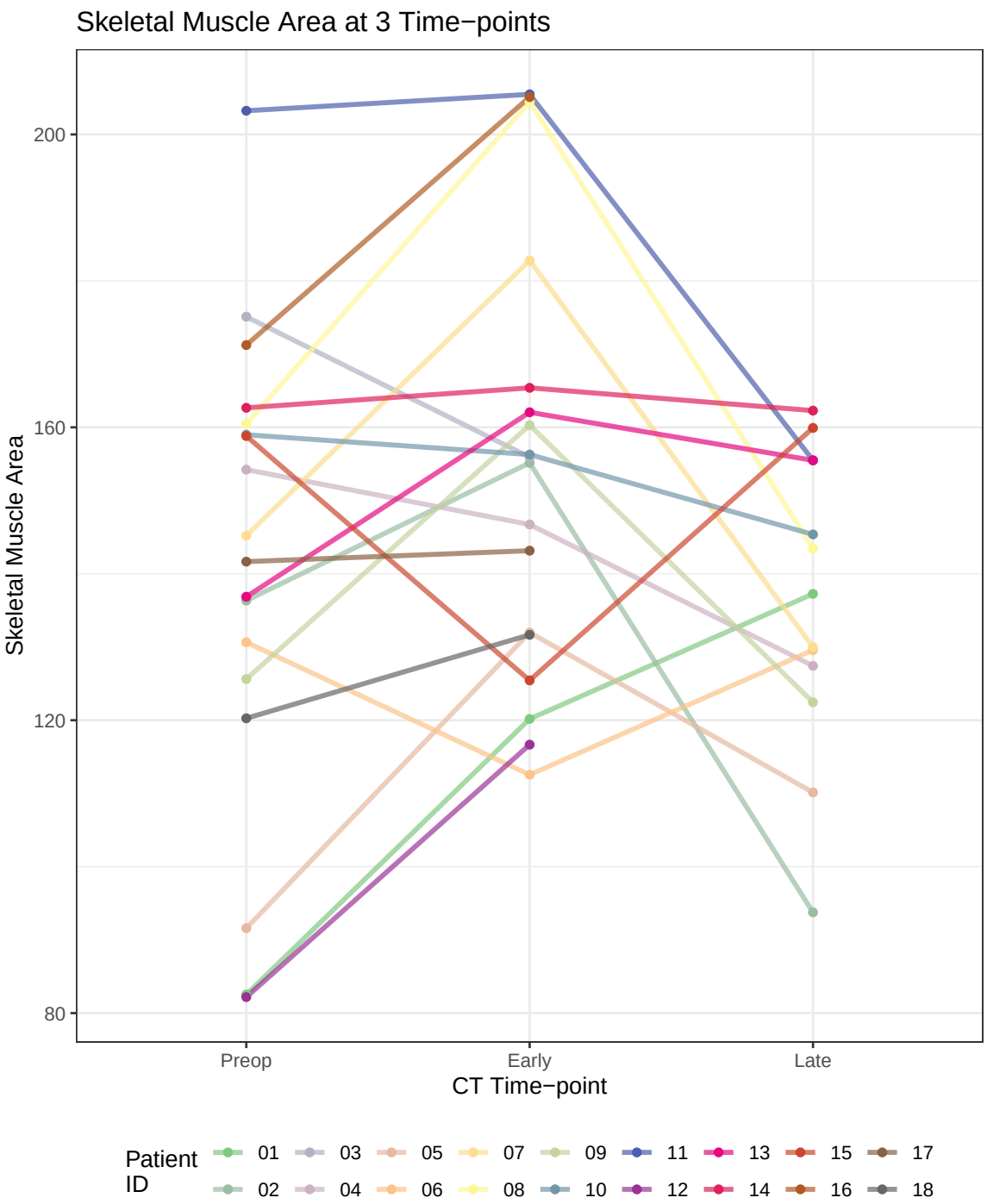



Figure 9.1: Timepoint Distribution of Skeletal Muscle Area

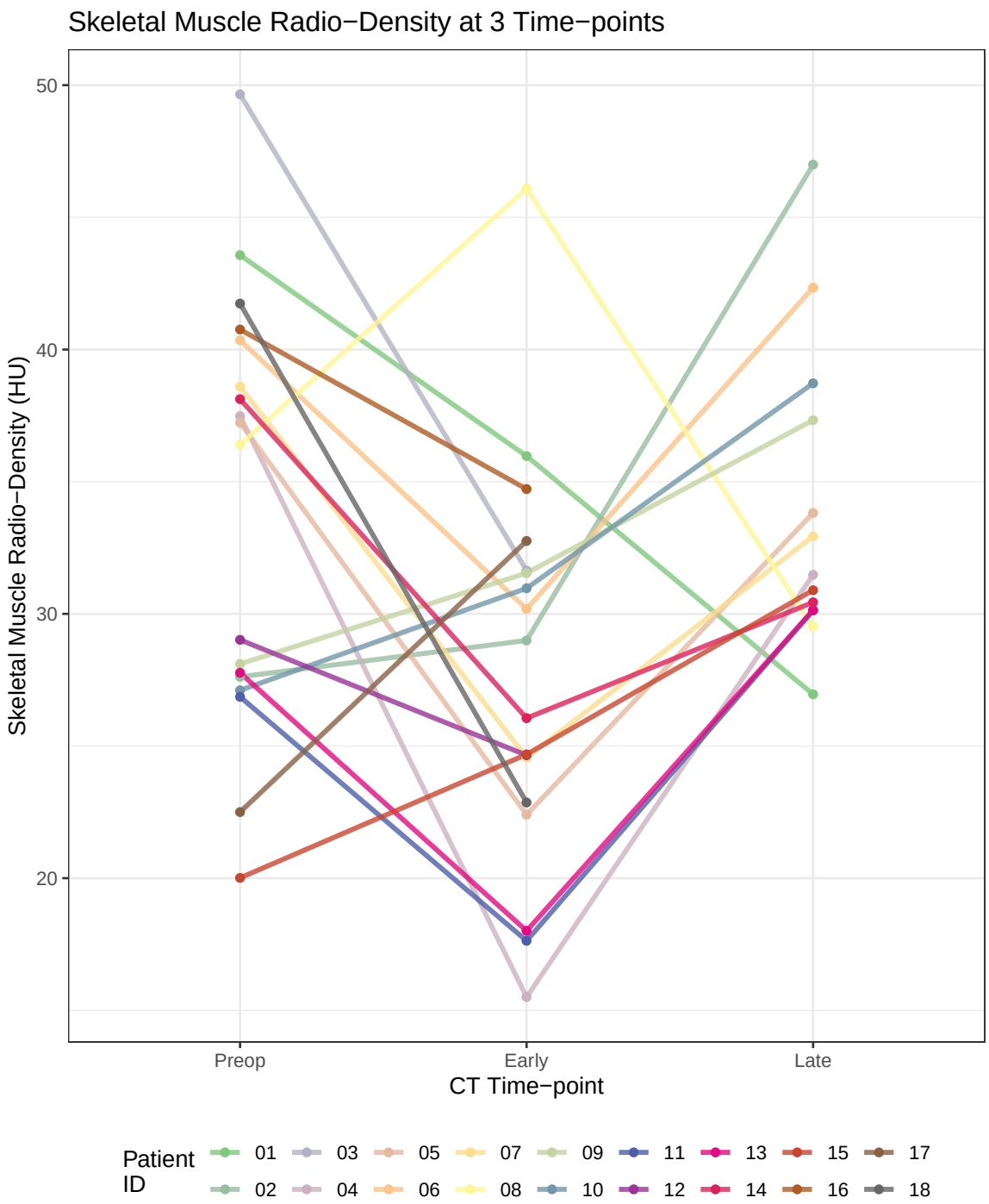



Figure 9.2: Timepoint Distribution of Skeletal Muscle Radio-Density

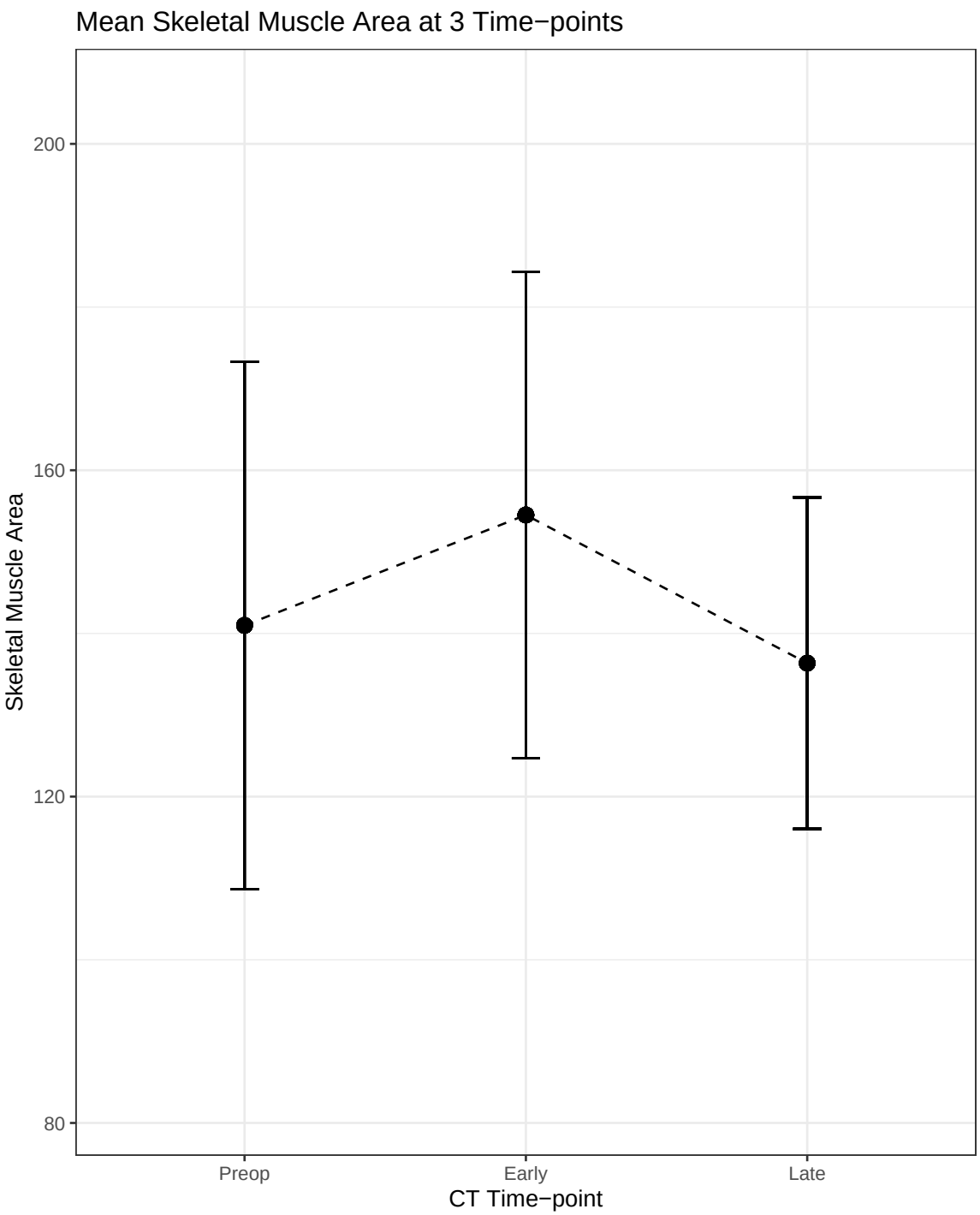



Figure 9.3: Mean and SD Timepoint Distribution of Skeletal Muscle Area

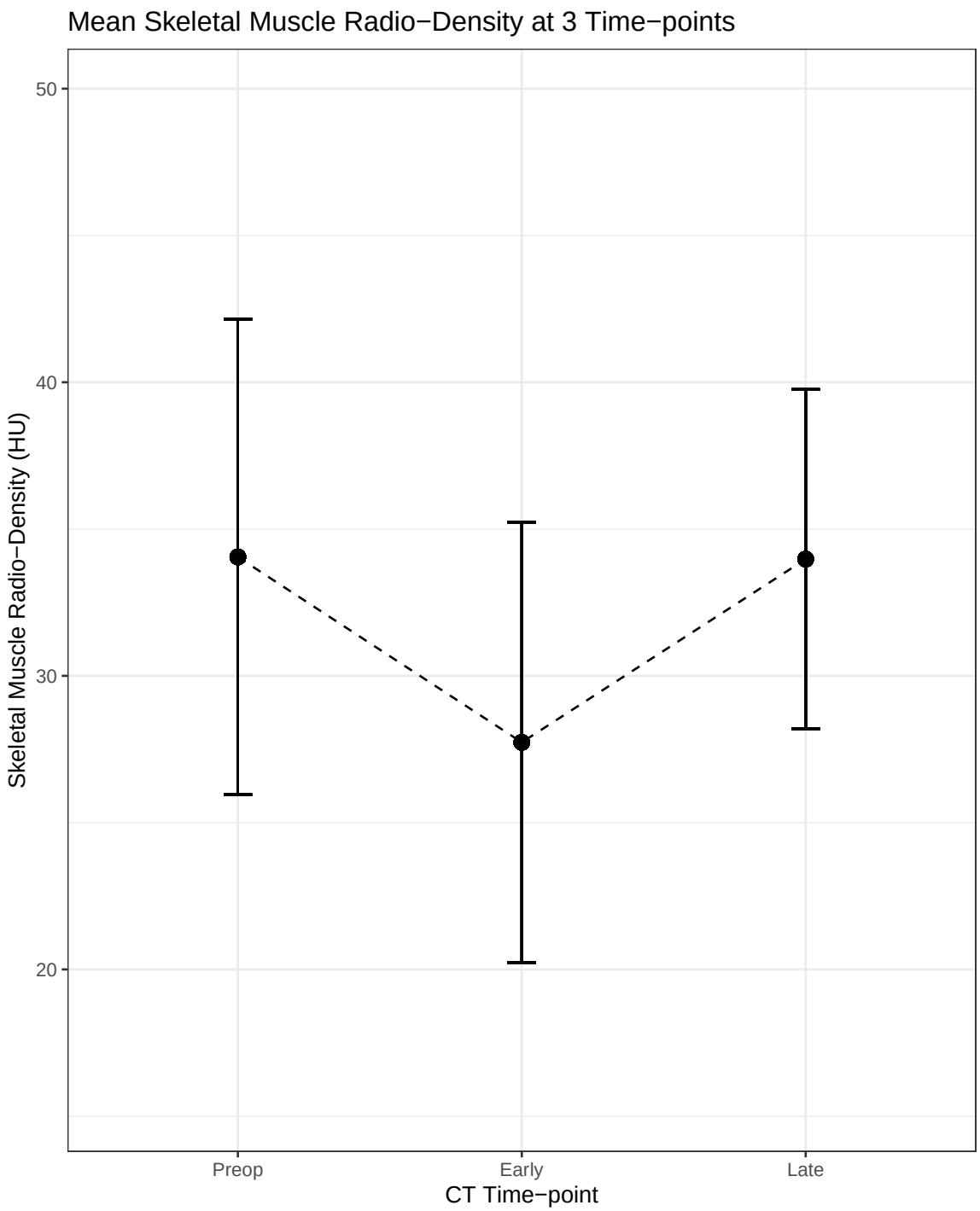



Figure 9.4: Mean and SD Timepoint Distribution of Skeletal Muscle Radio-Density

Table 9.2: Pairwise t-tests of CT SMA Variables by Time-point

| CT Time-point 1 | CT Time-point 2 | p value |
|---|---|---|
| Preop | Early postop | 0.08 |
| Preop | Late postop | 0.46 |
| Early postop | Late postop | 0.09 |

Table 9.3: Pairwise t-tests of CT SMD Variables by Time-point

| CT Time-point 1 | CT Time-point 2 | p value |
|---|---|---|
| Preop | Early postop | 0.04 |
| Preop | Late postop | 0.73 |
| Early postop | Late postop | 0.05 |

## 9.4 Discussion Analysing Relationship Between CT-BCA and Inflammation

The current study shows that in an ILOG cohort, body composition variables change between preoperative and post-complication CT scans in the context of severe SI. The cause for these changes are likely to differ between the measured variables in question; for example, although fat was not measured, a theoretical alteration in visceral fat area is likely to be related to operative technique which includes greater omental fat attached to the stomach being pulled into the thoracic cavity. Conversely, subcutaneous fat area is unlikely to be affected by operative technique. Similarly, the increase in skeletal muscle area noted will likely represent tissue oedema in context of inflammation rather than a true increase in skeletal muscle bulk, and this is reflected further in the observed decreases in SMD. The relationship between SMI and severe inflammation has been seen before in colorectal cancer patients [166] though radio-density has not been investigated. Oedema is due to changes in capillary fenestration sizes in SI resulting in albumin leakage and associated differences in osmotic pressure leading to increased tissue water content. The initial response to trauma and surgery also includes secretion of hormones with multiple effects. The release of arginine vasopressin from the posterior pituitary affects the kidneys by promoting resorption of sodium and thus water. The secretion of renin results in an increase aldosterone again promoting sodium and water retention [166]. An alternative explanation for the observed reduction in SMD is altered distribution of intravenous contrast during SI. Whilst the scans examined were all performed in the portal venous phase to ensure comparable body composition variables were obtained [59], systemic effects of inflammation on gross and micro- circulation should be considered. In severe inflammation and in septic states, the myocardium is known to be depressed [176] which may result in differences between "non-inflammatory" and "inflammatory" scans despite careful contrast gating. Additionally, severe inflammation is known to influence the microvasculature of multiple tissues [166]. These microscopic effects may alter the tissue bed uptake of contrast media thus altering the radio-density of the tissues. Contrast distribution may be affected further by the intravascular volume status of the patient and the quality of medical resuscitation. Therefore, one possible confounder of the present study is the lack of information regarding the volaemic status of the patients at time of CT scanning. It has been shown that SMD does not closely correlate with intramuscular protein content as seen in Chapter 7, and this may be because SMD is influenced by other patient factors such as SI highlighting the difficulties of using SMD as an outcome measure in trials of cachexia therapeutics. At time of follow-up scan, it is notable that SMD had returned to levels comparable to those recorded preoperatively, and that SMI did likewise. This would suggest that these variables mirror the biphasic "ebb and flow" of SI, and that as the inflammatory state returns towards normal, the CT variables do likewise.

An explanation for this observation is the presence and then resolution of tissue oedema altering muscle volume and radio-density for the duration of the inflammatory response.

The alteration of CT-BCA variables in response to inflammation, and particularly the increase in SMI with inflammation, has important connotations in the field of cancer cachexia. The elevation of SMI in severe SI, may be one end of a continuum. This could translate to SMI appearing to be preserved at lower levels of SI such as those seen in cancer patients, when in fact their *true* SMI is falling. This may mean that those patents who on CT-BCA do not meet cachexia criteria are in fact cachectic if tissue oedema could be controlled for - their *"dry* *muscle mass"* is below the cachexia cut-point. This would suggest that ongoing SI at clinically relevant levels may firstly mask the development of cachexia by volume standards, and secondly may be detectable in the alteration of muscle radio-density which has been labelled *"myosteatosis"*. Accordingly, the findings seen and reported - namely the poor outcomes amongst patients with low SMD and even more so those with both low SMD and sarcopenia may represent a combination of increased oedema (giving *"myosteatosis"*) and significant loss of muscle volume (enough to reach"sarcopenia" criteria), thus partially explaining the deleterious effects observed in patients with both [68]. Accordingly, the degree of SI present in cancer patients at time of CT-BCA should be delineated and may in fact represent a more powerful prognostic component of global assessment than previously considered.

Given these findings, and the potential underlying pathophysiology, it could be suggested that SI in addition to CT-BCA, should form part of or at least inform the diagnosis of cachexia in cancer patients.

# Chapter 10

# Discussion

## 10.1 CT-derived Definitions of Musculature

As described in Chapter 5, past work has focussed on the use of CT cut-points which have been derived from populations including healthy and diseased participants. The evolution of these cut-points from the original definition, and the introduction of CT as a replacement for DEXA is common throughout the sarcopenia and cachexia literature. Latterly, some authors have been noting that different populations exhibit different musculature and different muscle bulk [125]. Taking this argument to its logical conclusion, some investigators are concerned that each study should define sarcopenia in the studied population [107].

This approach may be attractive, but will lead to more questions regarding what is meant by sarcopenia and how it should be defined. In taking the lowest 2.5% or 10% or 33% of the population under study and using them to define sarcopenia, the investigators will also be including patients with a disease process which has led them to study inclusion. Considering that the original definition was "2 standard deviations below that of *healthy*" appendicular muscle mass, using a definition measure constructed from *unhealthy* patients will merely select out those with the worst measures amongst the already deteriorating. This will result in excellent separation of Kaplan-Meier curves, but will not necessarily reflect the prevalence of sarcopenia in the disease population.

In the present study, it was possible to utilise those *selected healthy* patients undergoing live donor nephrectomy as part of a transplant programme. This allowed the comparison the cut-points derived historically with modern locally-sourced healthy patients. Additionally, this allowed local cut-points to be derived in a similar fashion to that done in the index study by Baumgartner *et al*[7].

### 10.1.1 *"Healthy Sarcopenia"*

The results shown in Chapter 5 include the derivation and application of de-novo cut-points from a *selected healthy* population. This produced some surprising results, particularly in the number of LDN patients who met the published criteria for sarcopenia. Whilst there will be sarcopenic individuals in any population, the proportion of such individuals found in the LDN population who met sarcopenia definitions was unexpectedly high. This being the case, the question has to be whether this proportion is the result of a cut-point which is incorrect. The cut-point may be incorrect due to the derivation being from an un-matched population (such as using Western European cut-points in South-East Asian cohorts), or it may be incorrect due to changing methodology of measurement. In the present study, however, the methodology used (as described in Chapter 3) is the standard technique described in the sarcopenia literature. Additionally, the reference population used

for the index definition paper and the subsequent DEXA to CT, and indeed the BMI-stratification papers, all used populations derived from Western Europeans.

From this, it may be that *"health"* includes a degree of sarcopenia. As measures of musculature are continuous variables with the cut-point placed arbitrarily across a defined level of variable, an alteration in the underlying population will lead to more or fewer being above or below the line. It may be that the population attending the centre in question has shifted towards a lower musculature and that this could explain the increase in *"healthy sarcopenia"*. In any case, using locally-derived sarcopenia definitions as described by Baumgartner *et al* (specifically 2 standard deviations below the mean of a healthy population) results in a cut-point below which very few patients fall. This means that either the population are not sarcopenic and by extension not cachectic, or this method of producing a cut-point is not accurate for CT-BCA.

### 10.1.2 *"Non-Cancer Cachexia"*

The international consensus defining cachexia specifically relates to cancer, and the definitions used from this apply most strongly to the UGIC patients recruited for the present study. What is interesting to note, however, is that there are some patients who fall under the *"cachectic"* umbrella definition who do not exhibit evidence of cancer. The concept of other diseases causing cachexia is not new, and indeed both renal and cardiac cachexia are recognised, as is cachexia from chronic severe respiratory disease. What has not been widely described is that of a vascular aneurysmal disease causing cachexia. Clearly mesenteric ischaemia will lead to cachexia from anorexia and malabsorption. Thoracic and abdominal aortic aneurysmal disease is not known to exhibit such a phenotype, however.

The present study reveals that there is a non-negligible proportion of patients with aortic vascular disease who fall into cachexia definitions, leading to the question of whether this disease *can* lead to cachexia or whether the cachexia definition needs to be revised, perhaps on a population-specific basis as above.

## 10.2 Using CT to Subdivide QoL Metrics

Although published CT-BCA cut-points may result in unexpected classification of patients, nevertheless the cut-points may be useful for interpreting QoL metrics. Ideally, CT cut-points would allow an easy separation of patients into those with preserved, and those with deterioration in QoL.

### 10.2.1 QoL metric choice

The metrics chosen were the ones which were felt to be most appropriate for the population under study - specifically the UGIC population. Whilst additional QLQ-C30 cachexia bolt-on questionnaires have been developed, these were not available at the time of study design. The other questionnaires, and indeed the base QLQ-C30, were chosen to reflect a broad spectrum of general quality of life without being overly onerous to complete in a brief clinic visit. These metrics, although including response levels which should capture any *"non-normal"* response, are nevertheless primarily aimed at patients with cancer, and appear specifically to be aimed at those cancer patients with more advanced disease.

We were unable to find questionnaires which were aimed at both healthy and cancer patients, which were sufficiently slimline as to be acceptable to add-on to a brief clinic visit. This may represent a significant gap in the QoL toolbox but conversely it may be that there are few studies investigating those patients with generally preserved function and QoL or their healthy counterparts.

### 10.2.2 Absence of Relationship Between CT-BCA and QoL

The paucity of domains reaching both clinical and statistical significance across CT-BCA muscle variables, as described in Chapter 6, is in itself fairly interesting.

Hypotheses relating CT-BCA to clinical outcomes as demonstrated by Martin *et al*, by Mourtzakis *et al*, and by Rollins *et al*, suggest CT can detect muscular changes which influence or signpost prognosis. Were this the case, it would seem logical to suppose that a measurable deterioration in muscle composition should translate to a measurable deterioration in those QoL domains most closely related to muscular functioning. In the present study, we were unable to clearly demonstrate this relationship.

The explanation for this could come from multiple sources.

Firstly, it could be that there is no real relationship between muscle bulk and muscle function. This is an attractive hypothesis when considering real-world examples in the realm of athletics. Champion weight-lifters,

and champion long-distance runners are highly-trained, and highly-functional individuals. Both phenotypes compete internationally, and both undergo years of physical preparation and physique adjustment through training to reach the peak of their sports. Both have very different amounts of muscle, however, and by comparison with the weight-lifters the runners could be considered sarcopenic. This is clearly an extreme example, and serves to highlight and perhaps explain the apparent disconnect between muscle size and function, particularly with regard to QoL measures which as discussed above may not be sufficiently sensitive to detect subtle changes in a pre-operative population.

Secondly, it may be that there is in fact a relationship between muscle size and function, but there is a threshold above which evolved resilience and redundancy compensate for slow deterioration. This could possibly explain why there are some patients who have preserved function and QoL despite having very low volumes of muscle, and why those patients below these thresholds deteriorate rapidly. It may also be the case that true muscle deterioration is masked by systemic inflammation on CT as discussed in Chapter 9.

Thirdly, and with regard to muscle radio-density, the constituent parts of muscle which give rise to radio-density are imperfectly understood, as discussed in Chapter 7 and Chapter 8. Thus an attempt to explain differences in QoL by coercing these CT-BCA variables (specifically SMD) into binary categories may be unhelpful. Unless the cut-point used to define the categories is carefully chosen, any subtle differences in QoL will be lost.

Fourthly, it maybe that there were no real differences in QoL across the population of preoperative patients. This was certainly the case for the LDN cohort, as could be expected in a healthy population. There were differences when considering the AAA and UGI cohorts but those which reached both clinical and statistical significance were difficult to explain. This could be because there is a rational explanation which we have yet to discover, or because this in fact represents a Type II error, and a larger sample size would eliminate the differences.

### 10.2.3 Function and QoL

The differences within each cohort when split by function-related PROMs appear much more marked, and suggest that these may be more useful to characterise QoL within each group. What needs to be remembered, however, is that these PROMs are also in part QoL assessments in their own right. Accordingly, despite the attractive appearances of the data, what is being measured is one QoL PROM stratified by another.

Looking into more objective functional assessments such as gait speed, using these PROMs does not allow significant separation between groups. Again, this is not particularly surprising given the population under

study. Patients who met inclusion criteria were those who were sufficiently physically fit to undergo major surgery, and so their objective functioning by definition will be preserved.

### 10.2.4 QoL Measurement

Clearly the measurement and quantification of patient-reported QoL is important to assist in decision-making both for the physician and for the patient. The ability to understand objective measures which may influence QoL is a worthwhile target for research, and is one which should be pursued. The current difficulty is in not knowing either the relationship between CT-BCA variables and muscle function, or the relationship between muscle function and QoL. Closer investigation of these relationships will improve accuracy of measurements and should lead to better prediction of QoL in the future.

## 10.3 Relationship Between CT and Muscle Biochemistry

Having considered the importance of defining the relationship between CT and muscle constituents above, it then follows that an investigation should be conducted into this. Ideally, the use of CT-BCA variables would allow researchers to subdivide cohorts with carry-through into muscle biochemical distributions.

### 10.3.1 Relationship Between CT and Muscle Protein

As discussed in Chapter 7, both CT-BCA and muscle protein content were analysed in each cohort.

One major advantage of the present study is the quantification of the protein content of *"normal, healthy"* muscle. Analytical techniques have moved on from the 1950s and 1960s when *"reference man"* was defined. Concerns regarding the differences between these original and more modern techniques are discussed in Chapter 7 but the fact remains that the present study contains a worthwhile analysis of muscle protein content in a healthy cohort.

The values gathered for this protein content are remarkably similar to those measured in the previous millennium, and despite concerns regarding differing methodology the spread of protein content values in this larger cohort encompassed those of history.

Attempts to relate CT-BCA to these muscle protein content values proved less fruitful than hoped, particularly with regard to radio-density. It had been hypothesised that protein formed a sufficiently significant part of the muscle fibre that alterations in the protein content would be reflected in alterations in muscle radio-density. The absence of such a relationship would tend to suggest that there in another component to the cell which lends a greater radio-density or -lucency and which is not related to protein content. The lack of difference in protein content when patients were stratified by CT-BCA cut-points or by the international consensus definition would also suggest that clinically important differences or changes in patient phenotyping are not reflected in muscle protein content and that there is another component which remains to be measured and defined.

### 10.3.2 Relationship Between CT and CSA

As discussed in Chapter 8, and following on from the discussion above regarding a missing component of the cell, another hypothesis to explain differences in muscle radio-density was that the cell wall lent more to radio-density than protein content. The supposed mechanism behind this was that a larger number of smaller cells would proportionally have a greater amount of cell wall per unit area than a smaller number of larger cells.

Accordingly, if this were to be the case, explanations for functional outcomes could be built from this.

The use of a weighted mean to reduce very minute data to a single number was a technique which was used to allow testing between groups and to allow regression analysis at the cost of data ganularity. Alternative analysis techniques are, however, not suitable for analysing the quantity and type of data generated by the semi-automated CSA measurement equipment.

Analysis using the weighted mean did not reveal significant relationships between CSA and CT-BCA variables. This suggests that the cell wall as an explanation for muscle radio-density is not the final story and that there may be yet another reason for muscle to have greater or lesser radio-density, as discussed in Chapter 7 and Chapter 8.

### 10.3.3 CT and Biochemistry

Basic biochemical analyses of muscle samples obtained from healthy, cancer, and non-cancer populations have revealed values which are comparable to those of history. Additional analysis reveals interesting distributions of muscle cell size but does not fully explain a relationship between CT and the aspects of muscle biochemistry studied.

## 10.4 Relationship Between CT and Systemmic Inflammation

The analysis conducted in Chapter 9 reveals an additional facet to consider in the complex interaction between CT and muscle composition.

The severe inflammation triggered by anastomotic breakdown in oesophagetomy is marked but settles with resolution of the leak and with time. As diagnosis is often by CT, this allows an excellent opportunity to examine the changes in body composition brought about by systemic inflammation in a cancer population similar to that in the wider study cohort. The changes noted in CT-BCA across the time between the pre-operative scan and the post-operative, post-leak scan are marked.

One attractive hypothesis to unify the CT-BCA changes combines knowledge of the physiology of inflammation with observed differences in CT-BCA. With an early upsurge in inflammatory markers including CRP, comes a corresponding retention of water manifesting as tissue oedema. This oedema results in the an increase in tissue volume, and a trend for the radio-density of the tissue towards that of water. This nicely matches the observed changes in SMI and SMD. An increase in SMI between the pre-operative and post-leak scans was seen, which returned towards the pre-operative level in the late scan. Concurrently, a reduction in SMD between the pre-operative and post-leak scans was seen, which again returned towards the pre-operative level in the late scan. Both these changes, and their resolution, match the phases of systemic inflammation.

In examining the anastomotic breakdown population, the study was able to demonstrate clearly the effect of systemic inflammation on CT-BCA variables. Translating these changes to the UGIC population, and to cancer populations in general, would lead to the suspicion that systemic inflammation as previously observed will have a marked effect on CT-BCA in those patients.

It may be that SMI in cancer patients is thus elevated by tissue oedema in the presence of systemic inflammation, with concurrent reduction in SMD. This in turn would suggest that there is a population of patients who appear to have preserved muscle mass but in whom this may in fact be artificially elevated by oedema. Additionally, this population would have concurrently reduced SMD. This may in part explain the noted deleterious additive effects of reduced SMI with reduced SMD noted in a palliative pancreatic cancer population [68].

Indeed, the findings are important in the field of cachexia in that they suggest that without a knowledge of the state of systemic inflammation of the patient, true cachexia may be masked by tissue oedema, and that this underlies the apparent effects of reduced SMD.

# Chapter 11

# Conclusion

CT-BCA alone may not be sufficiently reliable to be used alone as an outcome measure for intervention trials purporting to influence patient function or quality of life.

- CT-BCA cut-points are not shown in this analysis to readily translate between populations.
- CT-BCA variables are not shown in this analysis to accurately predict skeletal muscle protein content.
- CT-BCA variables are not shown in this analysis to accurately predict other measures of muscle biochemistry such as cell CSA.
- CT-BCA variables are not shown in this analysis to accurately predict objective patient physical function.
- CT-BCA variables appear to have little impact on preoperative patient QoL in selected patients, in the current analysis.
- CT-BCA variables appear to be significantly influenced by SI and thus it would seem reasonable to attampt to avoid interpretation in isolation.

The current study investigated the relationships between CT-BCA variables and other components of patient assessment in an attempt to clearly define a linked pathway between what can be measured by CT and clinically relevant facets of patient life in cancer and cachexia. The study also attempted to underpin the values obtained from CT scans with biochemical explanations for each finding.

The application of CT-BCA cut-points derived in a North American population to a Scottish population including Cancer, Vascular, and Healthy Kidney Donor patients results in an expected prevalence of cachexia and sarcopenia in the cancer group, but a much increased prevalence of sarcopenia in the non-cancer groups and a significant proportion of vascular patients meeting cancer cachexia diagnostic criteria. The results from this suggest that these cut-points may not be as clear-cut as previously considered. The results also point to a hitherto unrecognised facet of vascular disease, specifically weight loss. It may be that these patients are presenting with more advanced conditions than the pre-operative cancer patients but in a group without a known weight losing condition, this is important to recognise.

Investigating the relationship between CT-BCA and muscle biochemistry should have yielded more positive results. The suspicion was that protein content gave some degree of radio-density to skeletal muscle. The lack of relationship between protein content and muscle radio-density was surprising. Looking further into other

aspects of muscle biochemistry in the form of CSA might have been expected to reveal a relationship with muscle mass, or with muscle radio-density. The absence of such a relationship would suggest that there are other components to account for variation in SMD and that looking to CT as a means to infer muscle protein content is flawed.

If CT-BCA variables struggle to classify patients into sarcopenic and non-sarcopenic, and show little relationship with biochemical components of muscle, perhaps they may be of use in predicting patient function and QoL as it is known that CT-BCA variables have a relationship with poor outcome? Again, however, the relationship was not to be found. With regards to physical functioning and in particular gait speed, it may be that the population under study is not the correct one as by their nature these patients must have preserved function. When considering QoL, however, it would be expected that those with worse QoL would be likely to have more advanced disease, and thus would be identifiable by CT-BCA. Again, however, this was not shown to be the case. It appears that CT-BCA variables are unable to predict changes in QoL with any strength. Once again this could be because of the patient group selected having preserved QoL or because the subtle differences in QoL are not captured by the questionnaires used, however attempts to draw conclusions about QoL from CT-BCA should be done with care.

The relation of CT-BCA and conclusions based on these variables with outcomes can be visually summarised in Figure 11.1. Accordingly, a reconsideration of the relationship between muscle mass on CT and function must be considered. Rather than a linear relationship, a more complex series of curves may link mass and function, with sharp deterioration in function following the arrival at a critical level of skeletal muscle mass. This is illustrated in Figure 11.2, and together with the findings demonstrated in the present study to date could help to inform future cachexia study design and recruitment.

Both of these figures, it has to be remembered, are based upon the findings of this study and as such there may well be other aspects of the relationship between CT-BCA and cachexia which are not tested and which may influence the interpretation of the study findings when taken in a wider context. Whilst the study has been unable to substantiate links between SMI, SMD, and QoL/PF etc., nevertheless it is likely that there is a relationship between muscle mass and function, possibly more marked at the extremes of the curves. It may be that the x-axis (marked "muscle mass") may require re-labelling to another measure of muscle, such as e.g. "quality", though what this represents and how it should be measured (whether a new measurement entirely, or some combination of current measures) is uncertain at present.

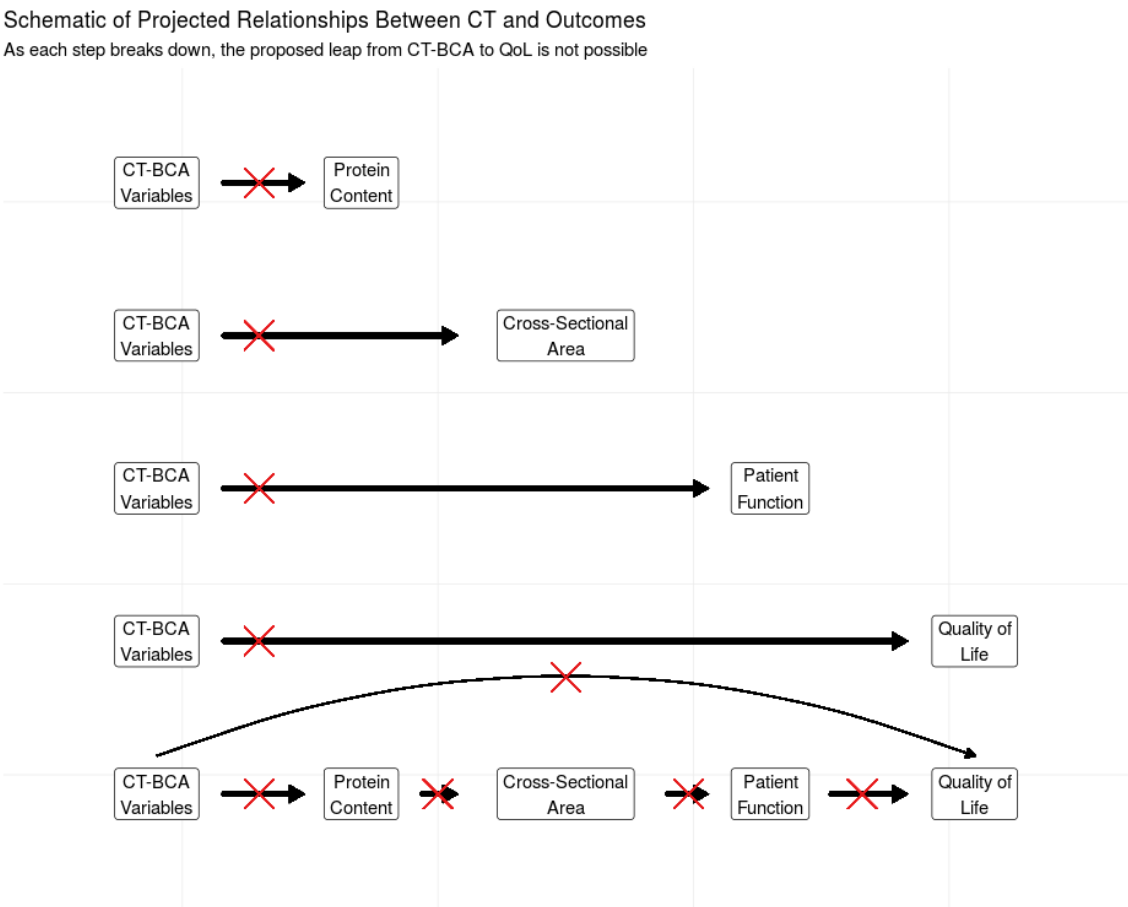



Figure 11.1: Relationship Between CT and Outcomes

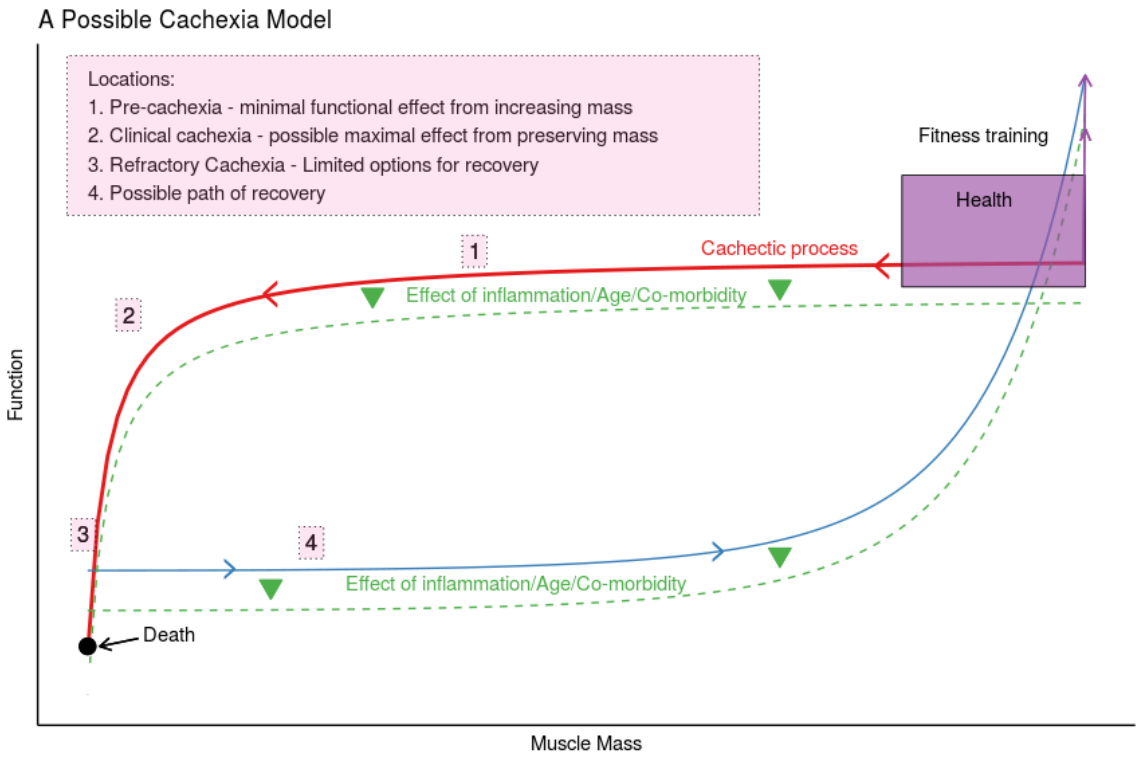



Figure 11.2: Proposed New Cachexia Model Linking Muscle Mass and Function

## 11.1 Future Directions

### 11.1.1 Body Composition and Muscle Biochemistry

The increasing sophistication of analysis packages for interrogating cross-sectional images gathered as part of routine clinical care, together with the increased availability of such imaging in routine clinical care should generate large quantities of data for analysis. Whilst this may take some time before it could be classified as *"big data"*, nevertheless a combination of a minimum agreed dataset with an international collaborative effort may result in a very powerful tool for research and guiding future efforts.

Another technique for measuring skeletal muscle which is being reported at present is the use of $D_3$-Creatine dilution. The technique is described by Shankaran *et al* [177] and involves the administration of a single dose of tracer, followed by a fasted urine sample at a future date. The authors showed a strong correlation with bioimpedence spectroscopy and propose this as a simple, rapid method for measuring muscle mass. Indeed, this technique has been used in a large cohort study of male ageing [178], and has shown strong associations with clinically relevant outcomes in older males. Such a technique is clearly of more use in research than in widespread clinical practice, and may be more onerous for patients than undergoing their routine imaging. Nevertheless, a replication of the current study with the addition of this tracer may allow greater analysis and interpretation of both CT-BCA and muscle biochemistry.

Advances in CT-BCA interpretation, and increased usage of MRI scanning should increase the amount of data available for interpretation. It will be interesting to see how these techniques relate to physiological and biochemical measures - both at the cellular and at the whole body level.

### 11.1.2 Quality Of Life and Function

Currently-used quality of life measures are not aimed at patients with preserved QoL and function. Whilst there are QoL tools aimed at patients suffering cachexia these are undergoing validation, and it is unclear how much better they would be in a comparable cohort to the current study.

In a similar fashion, tools used to measure function objectively are likely to be of limited sensitivity for detecting changes in a selected population with preserved function. The prevalence of reduced function in a preoperative cohort is small, and thus the chance of a test correctly identifying this must be interpreted accordingly.

This is not to say that such measures should be ignored - rather that either more sensitive tests should be developed to capture subtle changes, or that the outcome of these tests should be interpreted as a continuum

and as part of a wide-ranging assessment rather than a binary set.

# Appendix A

# Published Work

## A.1 Articles

### A.1.1 The relationship between muscle protein content and CT-derived muscle radio-density in patients with upper GI cancer

Michael I Ramage, Neil Johns, Christopher D. A. Deans, James A. Ross, Thomas Preston, Richard J. E. Skipworth, Carsten Jacobi, Kenneth C. H. Fearon

Clin Nutr. 2018 Apr;37(2):752-754. doi: 10.1016/j.clnu.2016.12.016. Epub 2016 Dec 27.

### A.1.2 The relationship between muscle mass and function in cancer cachexia – smoke and mirrors?

Ramage MI, Skipworth RJE

Curr Opin Support Palliat Care. 2018 Dec;12(4):439-444. doi: 10.1097/SPC.0000000000000381.

## A.2 Abstracts

### A.2.1 *Presented at SCWD Paris 2015 in Poster Form*

#### A.2.1.1 Low muscle attenuation correlates with protein levels in patients with GI cancer

Ramage M, Johns N, Skipworth R, Deans C, Jacobi C, Fearon K

### A.2.2 *Presented at SCWD Berlin 2016 in Poster Form*

#### A.2.2.1 The influence of operative stress, systemic inflammation and sepsis on CT body composition variables

Michael Ramage, Graeme W Couper, James A Ross, DA Christopher Deans, Richard JE Skipworth, Kenneth CH Fearon (Deceased 3rd September 2016)

#### A.2.2.2 The relationship between PET-CT-derived measurements of muscle glucose metabolism and myofibrillar protein synthesis in oesophageal cancer patients.

Michael Ramage, Alisdair J MacDonald, Dilip Patel, DA Christopher Deans, James A Ross, Tom Preston, Richard JE Skipworth, Ken CH Fearon (deceased Sept 3rd, 2016)

### A.2.3 *Presented at SCWD Rome 2017 in Poster and Oral Form*

#### A.2.3.1 Low muscularity by CT definition is prevalent in both cancer and non-cancer control populations

Michael I Ramage, Aleksandra Staniszewska, Edi Schuepbach, Ronnen Roubenof, Gabriel Oniscu, Stephen J Wigmore, DA Christopher Deans, James A Ross, Carsten Jacobi, Richard JE Skipworth

#### A.2.3.2 The emerging disconnect between muscle mass and function: evidence from different patient populations

Michael I Ramage, Janice Miller, Edi Schuepbach, Ronnen Roubenof, DA Christopher Deans, Stephen J Wigmore, James A Ross, Carsten Jacobi, Richard JE Skipworth

#### A.2.3.3 Patient-reported quality of life has no clear relationship with CT-derived body composition

Michael I Ramage, Edi Schuepbach, Ronnen Roubenof, DA Christopher Deans, Stephen J Wigmore, James A Ross, Carsten Jacobi, Richard JE Skipworth

# Appendix B

# Published Manuscripts

Clinical Nutrition 37 (2018) 752–754

Contents lists available at ScienceDirect

Clinical Nutrition

journal homepage: http://www.elsevier.com/locate/clnu

Short Communication

# The relationship between muscle protein content and CT-derived muscle radio-density in patients with upper GI cancer

Michael I. Ramage [a, *], Neil Johns [a], Christopher D.A. Deans [a], James A. Ross [a], Thomas Preston [b], Richard J.E. Skipworth [a], Carsten Jacobi [c], Kenneth C.H. Fearon [a, 1]

[a] Department of Clinical Surgery, Royal Infirmary of Edinburgh, 51 Little France Crescent, Edinburgh, EH16 4SA, Scotland, UK
[b] Stable Isotope Biochemistry Laboratory, Scottish Universities Environmental Research Centre, Rankine Avenue, Scottish Enterprise Technology Park, East Kilbride, Glasgow, G75 0QF, Scotland, UK
[c] Musculoskeletal Diseases Area, Muscle Group, Novartis Pharma AG, Novartis Campus, WSJ-152.2.72.04, CH-4056, Basel, Switzerland

ARTICLE INFO

*Article history:*
Received 1 September 2016
Accepted 19 December 2016

*Keywords:*
Body composition
Cachexia
Cancer
Imaging analysis
Protein content
Skeletal muscle

SUMMARY

*Introduction:* Cancer cachexia is a multifactorial syndrome characterized by skeletal muscle loss. Cross-sectional analysis of CT scans is a recognized research method for assessing skeletal muscle volume. However, little is known about the relationship between CT-derived estimates of muscle radio-density (SMD) and muscle protein content. We assessed the relationship between CT-derived body composition variables and the protein content of muscle biopsies from cancer patients.

*Methods:* Rectus abdominis biopsies from cancer patients (n = 32) were analysed for protein content and correlated with phenotypic data gathered using CT body composition software.

*Results:* Skeletal muscle protein content varied widely between patients (median μg/mg wet weight = 89.3, range 70–141). There was a weak positive correlation between muscle protein content and SMD (r = 0.406, p = 0.021), and a weak positive correlation between protein content and percentage weight change (r = 0.416, p = 0.018).

*Conclusion:* The protein content of skeletal muscle varies widely in cancer patients and cannot be accurately predicted by CT-derived muscle radio-density.



## 1. Introduction

Cancer cachexia has been defined as a multifactorial syndrome characterized by ongoing loss of skeletal muscle mass that cannot be fully reversed by conventional nutritional support [1]. Cachexia affects the majority of patients with advanced cancer and is associated with a reduction in treatment tolerance, response to therapy, quality of life and duration of survival. The assessment and classification of cachexia is important for patient prognostication and treatment guidance. Modern cross-sectional imaging techniques, combined with software planimetry, have been proposed as one method for the assessment of skeletal muscle and fat mass in cachectic patients. In this technique, the anatomical limits of different tissue areas are defined by radio-density (measured in Hounsfield units (HU)). Lean mass (LM) and total fat mass can then be predicted by calculating skeletal muscle and fat areas at the level of the third lumbar vertebra (L3), and extrapolating these values to the whole body by using validated regression equations [2]. Increased fat infiltration of skeletal muscle (myosteatosis) results in lower skeletal muscle radio-density (SMD) on CT [3].

For cancer patients, image analysis can be performed on CT scans undertaken as part of the patient's routine staging investigations. Low skeletal muscle index (SMI, defined as skeletal muscle area normalized for height in $m^2$ [4]), as assessed by CT, has been shown to be associated with poor prognosis in a wide range of solid epithelial malignancies. Moreover, reduced SMD is also thought to be an independent predictor of adverse outcome in respiratory and GI cancer patients [5]. However, it is not known whether reduced SMD may also reflect variation in skeletal muscle protein content. We aimed to ascertain whether body composition variables measured by standard L3 CT scan analytic software are

* Corresponding author.
*E-mail addresses:* mramage@exseed.ed.ac.uk (M.I. Ramage), neiljohns@doctors.org.uk (N. Johns), chris.deans@nhslothian.scot.nhs.uk (C.D.A. Deans), j.a.ross@ed.ac.uk (J.A. Ross), tom.preston@glasgow.ac.uk (T. Preston), richard.skipworth@nhslothian.scot.nhs.uk (R.J.E. Skipworth), carsten.jacobi@novartis.com (C. Jacobi).
[1] Deceased.

http://dx.doi.org/10.1016/j.clnu.2016.12.016
0261-5614/

related to the measured protein content of skeletal muscle biopsies in humans.

## 2. Methods

Patients undergoing surgical resection for upper gastrointestinal cancer (4 gastric, 12 oesophageal, 3 junctional, 12 pancreatic and 1 duodenal) were recruited. Patients were defined as weight-losing if their weight at diagnosis was >5% less than their pre-illness stable weight [1]. Sarcopenia was defined using SMI cut-offs according to BMI [4]. SMD was measured between −30 and 150 HU, with low SMD defined as <41 HU with BMI <25 and <33 with BMI >25 [4].Routine blood sampling was performed, including plasma C-reactive protein (CRP) levels measured by automated turbidimetry. Preoperative staging CT scans were analysed at L3 level using semi-automated Slice-O-Matic software v4.2 (Tomovision Montreal-Canada), which defines area measurements of skeletal muscle, subcutaneous fat, and visceral fat.

Under general anaesthesia during resectional surgery, rectus abdominis muscle biopsies were taken, snap-frozen in liquid nitrogen, and stored at −80 °C in monitored freezers prior to analysis. These biopsies were pulverized and weighed using an analytical balance (Mettler Toledo), then lysed with Phosphosafe Extraction Reagent (Merck Millipore, Billerica, MA, USA) before being homogenized and centrifuged. The supernatant was analysed for total soluble protein (including myofibrillar and cytosolic) content using commercially available BCA protein assay kits (Pierce Biotechnology, Thermo Fisher Scientific, Rockford, IL) [6].

Data were analysed using descriptive statistics. For variables of interest, correlation analysis was performed by non-parametric Spearman's rank coefficient, and comparison of groups was by Mann–Whitney U-test using IBM SPSS Statistics (IBM Corp. Released 2013. IBM SPSS Statistics for Windows, Version 22.0. Armonk, NY: IBM Corp.). Microsoft Excel was used for graphical analyses. Ethical approval for this study was granted by the local Research Ethics Committee.

## 3. Results

Thirty-two (n = 32; M:F 26:6) cancer patients (median age 64.5 years, range 43–83) were included. 17 (53.1%) were weight-stable and 15 (46.9%) were weight-losing. Two patients had Stage 1 disease, 6 had Stage 2 disease, 15 had Stage 3 disease, 5 had Stage 4 disease, and in 4 patients disease stage was not recorded. By pre-established CT criteria [4], 19 (59.4%) were sarcopenic and 13 (40.6%) were not sarcopenic; 25 (78.1%) had normal SMD and 7 (21.9%) had low SMD (related to myosteatosis).

Results are shown in Table 1. Median skeletal muscle protein content (μg/mg wet weight) varied significantly between cancer patients, and was significantly lower in the weight-losing patients compared with the weight-stable group (88.1 range (71–97) versus 92.3 (83–141) p = 0.027). There was a weak positive correlation between protein content and percentage weight change (r = 0.416, p = 0.018) (Fig. 1). However, muscle protein content was similar in sarcopenic and non-sarcopenic patients as defined by CT (88.3 (71–141) versus 89.7 (74–128), p = 0.79). Muscle protein content was lower in the "low SMD" group than the "normal SMD" group (median 93.1, range 71–141 versus 83.2, 74–90; p = 0.03), and there was a weak positive correlation between muscle protein content and SMD (r = 0.406, p = 0.021) (Fig. 1b). Muscle protein content did not correlate significantly with any other CT marker of body composition, including muscle area, stature-adjusted muscle area, visceral adipose tissue area, or subcutaneous adipose tissue area. Additionally, muscle protein content did not correlate significantly with age, weight, BMI, or plasma CRP.

SMD correlated negatively with age (r = −0.543, p = 0.001) and visceral adipose tissue area (r = −0.384, p = 0.04), but not levels of systemic inflammation as indexed by plasma CRP (r = −0.175, p = 0.337), suggesting that myosteatosis increased with age and visceral adiposity.

There was no significant difference in skeletal muscle radiodensity, skeletal muscle index, skeletal muscle protein content, visceral adipose tissue area, subcutaneous adipose tissue area, or percentage weight change across categories of cancer stage.

## 4. Discussion

In the present study, there was substantial variation in the skeletal muscle protein content of the cancer patients. The median level observed was reduced compared with values used to inform standard reference tables and reference man [7] (average 172 μg/mg wet weight; range 166.9–177.5; n = 3 healthy, young males). As such, the present findings are consistent with previous compartmental body composition of weight-losing GI cancer patients using measurement of whole body nitrogen, potassium and water [8]. The latter study identified that, compared with healthy controls, both muscle mass and protein content are reduced by >60% with a coefficient of variation for muscle protein content rising from 31% to 77% in the weight-losing individuals compared with the weight-stable controls [8]. Factors that might contribute to a reduced protein content in the wet weight of muscle from cancer patients include increased protein degradation, reduced protein synthesis, increased intramuscular fat (myosteatosis) [3], or the relative expansion of extracellular water space [9]. Alternatively, in comparison with reference man, there may be differences based on different methodologies: the colorimetric protein assay used in the present study is sensitive to certain amino-acid residues that may not be present in the same proportion in all tissues and, although precise, may not be as accurate as a physico-chemical assay for nitrogen as an index of protein mass. For example, the BCA technique relies on the reduction of $Cu^{2+}$ to $Cu^{+}$ by protein with secondary detection of the reduced copper; this renders it susceptible to the presence of other reductive agents present in the sample. Nitrogen assays, however, will be affected by non-protein nitrogen sources and also by non-muscle protein. Comparative studies in this field have been performed in the food industry but remain to be done in human skeletal muscle.

There was a weak correlation between muscle protein content and the degree of weight loss in the present study (Fig. 1b). A reduction in the myofibrillar content of muscle could explain

**Table 1**
Weight, weight change, CT body composition variables and protein content of rectus abdominis muscle biopsy of 32 patients with upper GI cancer.

| Variable | Median | Range |
|---|---|---|
| Premorbid stable weight (kg) | 81.6 | 57–137 |
| Premorbid BMI (kg/m$^2$) | 27.0 | 20.4–43.9 |
| Weight at time of biopsy (kg) | 75.3 | 52.4–133 |
| Percentage weight change (%) | −3.7 | −25 to +10.9 |
| L3 muscle area (cm$^2$) | 132.4 | 86.9–235 |
| Skeletal muscle index (cm$^2$/m$^2$) | 46.8 | 33.9–76.8 |
| L3 SMD (HU) | 39.2 | 25–56 |
| Muscle protein content (μg/mg wet weight) | 89.3 | 70–141 |
| Muscle protein content (weight-losing patients) (μg/mg wet weight) | 88.1 | 71–97 |
| Muscle protein content (weight-stable patients) (μg/mg wet weight) | 92.3 | 83–141 |
| Muscle protein content (low SMD patients) (μg/mg wet weight) | 83.2 | 74–90 |
| Muscle protein content (normal SMD patients) (μg/mg wet weight) | 93.1 | 71–141 |

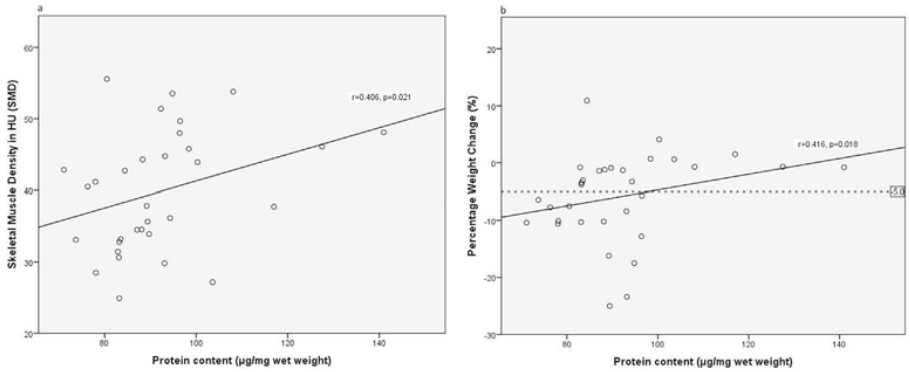



**Fig. 1.** Correlation between protein content of rectus abdominis muscle biopsy in 32 patients with upper GI cancer and skeletal muscle radio-density (a) or percentage weight loss (b). Dashed line represents 5% weight loss.

previous studies in cancer patients that have shown a reduction in muscle strength when expressed in relation to the cross-sectional area of the muscle [10]. Current intervention studies rely on cross-sectional imaging to look for a positive effect of anabolic agents on muscle, using volume as the index measure, with the assumption that the muscle protein content remains constant. The present results show substantial variation in muscle protein content and suggest that CT-derived muscle volume does not necessarily relate to muscle protein mass. That some weight-stable patients had biopsies that demonstrated a relatively low muscle protein content (Fig. 1b) may reflect the concept that in cachexia there may be a pre-cachectic state where metabolic change occurs ahead of weight-loss [1].

Limitations of the present study include the absence of healthy controls, the diverse patient cohort and the relatively small sample size. Also, although the protein extraction method allows measurement of total soluble (including myofibrillar) protein, it may not reflect total cellular protein, as the insoluble pellet remaining is likely to contain polymerized-protein filaments and some nuclear and organelle proteins. Additionally, the analytical CT software produces a mean HU measurement of all skeletal muscle present on a CT scan slice, whereas the biopsies represent only a small portion of one of the many different muscles present at L3.

Whereas weight-losing patients (i.e. cachexia) had much lower protein content than weight-stable individuals a similar pattern was not observed for sarcopenic versus non-sarcopenic individuals. Whilst weight-loss is a dynamic measure, sarcopenia may result from a variety of current and historical events and it would probably require a much larger cohort of patients to dissect out the difference.

### *4.1. Conclusions*

The present study suggests that, in cancer patients, muscle protein content varies widely and cannot accurately be predicted by CT image analysis.

## Statements of authorship

Concept and writing performed by MR, RS, NJ, CD, CJ, TP, JR, and KF.

Body composition analysis and interpretation by MR, NJ, TP, RS, and KF.

Muscle protein content assayed by CJ.

All authors critically revised the article for important intellectual content, and all approved the final submitted version.

## Funding sources

This study was supported by Novartis [PO number 3001673636], and by ESPEN [ESPEN Research Fellowship Grant 2015].

CJ is an employee of Novartis.

## Conflict of interest

All authors have completed ICMJE CoI declaration forms.

## Acknowledgements

Thanks go to the Tissue Injury and Repair Group, Novartis, NHS Lothian, and the University of Edinburgh.

## References

[1] Fearon K, Strasser F, Anker SD, Bosaeus I, Bruera E, Fainsinger RL, et al. Definition and classification of cancer cachexia: an international consensus. Lancet Oncol 2011 May;12(5):489–95.
[2] Mourtzakis M, Prado CMM, Lieffers JR, Reiman T, McCargar LJ, Baracos VE. A practical and precise approach to quantification of body composition in cancer patients using computed tomography images acquired during routine care. Appl Physiol Nutr Metab 2008 Oct;33(5):997–1006.
[3] Aubrey J, Esfandiari N, Baracos VE, Buteau FA, Frenette J, Putman CT, et al. Measurement of skeletal muscle radiation attenuation and basis of its biological variation. Acta Physiol 2014 Mar;210(3):489–97.
[4] Martin L, Birdsell L, MacDonald N, Reiman T, Clandinin MT, McCargar LJ, et al. Cancer cachexia in the age of obesity: skeletal muscle depletion is a powerful prognostic factor, independent of body mass index. J Clin Oncol 2013 Apr 20;31(12):1539–47.
[5] Prado CM, Lieffers JR, McCargar LJ, Reiman T, Sawyer MB, Martin L, et al. Prevalence and clinical implications of sarcopenic obesity in patients with solid tumours of the respiratory and gastrointestinal tracts: a population-based study. Lancet Oncol 2008;9(7):629–35.
[6] Johns N, Hatakeyama S, Stephens NA, Degen M, Degen S, Frieauff W, et al. Clinical classification of cancer cachexia: phenotypic correlates in human skeletal muscle. PLoS One 2014;9(1):e83618.
[7] Dickerson JWT, Widdowson EM. Chemical changes in skeletal muscle during development. Biochem J 1960 Feb 1;74(2):247–57.
[8] Cohn SH, Gartenhaus W, Sawitsky A, Rai K, Zanzi I, Vaswani A, et al. Compartmental body composition of cancer patients by measurement of total body nitrogen, potassium, and water. Metabolism 1981 Mar 1;30(3):222–9.
[9] Fearon KC, Preston T. Body composition in cancer cachexia. Infusionstherapie 1990 Apr:63–6.
[10] Stephens NA, Gray C, MacDonald AJ, Tan BH, Gallagher IJ, Skipworth RJE, et al. Sexual dimorphism modulates the impact of cancer cachexia on lower limb muscle mass and function. Clin Nutr 2012 Aug;31(4):499–505.

# The relationship between muscle mass and function in cancer cachexia: smoke and mirrors?

*Michael I. Ramage and Richard J.E. Skipworth*

**Purpose of review**
Randomized clinical trials of cancer cachexia interventions are based on the premise that an increase in the muscle mass of patients is associated with consequent improvements in muscle function, and ultimately, quality of life. However, recent trials that have succeeded in demonstrating increases in lean body mass have been unable to show associated increases in patient physical function. In this review, we examine the potential causes for this lack of association between muscle mass and function in cancer cachexia, paying particular attention to those factors that may be at play when using body composition analysis techniques involving cross-sectional imaging. Moreover, we propose a new population-specific model for the relationship between muscle mass and physical function in patients with cancer cachexia.

**Recent findings**
The ROMANA 1 and 2 trials of anamorelin (a novel ghrelin agonist) and the POWER 1 and 2 trials of enobosarm (a selective androgen receptor modulator) were able to demonstrate improvements in patient lean body mass, but not the functional co-primary endpoints of handgrip strength and stair climb power, respectively. We report similar confirmatory findings in other studies, and describe potential reasons for these observations.

**Summary**
The relationship between muscle mass and muscle function is complex and unlikely to be linear. Furthermore, the relationship is influenced by the techniques used to assess nutritional endpoints [e.g. computed tomography (CT)]; the nature of the chosen physical function outcome measures; and the sex and severity of the recruited cachectic patients. Such factors need to be considered when designing intervention trials for cancer cachexia with functional endpoints.

**Keywords**
body composition, cachexia, cancer, muscle

## INTRODUCTION

Regulatory agencies have previously stipulated that intervention trials in cancer cachexia should use nutritional and functional co-primary endpoints [1]. With this in mind, it would seem to make prima facie sense that nutritional outcomes that assess muscle size should correlate with muscle function in any case, at least in terms of explosive measures of function, such as strength and power. Weightlifters have large muscles by definition. However, the relationship between muscle mass and muscle function is more complex than would be expected at first glance. In contrast to weightlifters, marathon runners tend to be slighter in build with less pronounced musculature, and their performance is not linked with muscle mass [2]. It is arguably this latter endurance-type performance that would be of most clinical benefit to elderly patients with cancer cachexia. However, at the present time, there are no agreed recommendations or guidelines for physical function or nutritional outcome measures that should be assessed in trials of intervention for cachexia.

The use of muscle mass as a nutritional outcome measure [or lean body mass (LBM) as a surrogate outcome measure] appears to be relatively reliable in clinical trials looking to assess the effect of pharmacological interventions. To this end, the effect of anamorelin, an oral ghrelin receptor antagonist (Helsinn) was studied in a series of trials named ROMANA 1, 2, and 3. In ROMANA 1 and 2, a 12-week treatment period increased LBM [assessed by

Clinical Surgery, University of Edinburgh, Royal Infirmary of Edinburgh, Edinburgh, United Kingdom

Correspondence to Richard J.E. Skipworth, MBCHB, BSc, FRCSEd, MD, Clinical Surgery, University of Edinburgh, Royal Infirmary of Edinburgh, Edinburgh EH16 4SA, United Kingdom. Tel: +44 131 536 1000; E-mail: richard.skipworth@nhslothian.scot.nhs.uk

**Curr Opin Support Palliat Care** 2018, 12:439–444
DOI:10.1097/SPC.0000000000000381

## KEY POINTS

- There has been an unexpected inability of cachexia trials to demonstrate a related and synchronous increase in muscle mass and function.
- The best methodology for measuring body composition is still unclear, and the final chosen technique will influence any observed relationship with physical function.
- The identity of the best physical function outcome measure to relate nutritional outcome measures is unknown, and its relationship to body composition measures is unclear.
- There is likely to be an error in the understanding of the nature of the relationship between muscle mass and function, and we propose a new non-linear model.
- The true relationship between muscle mass and function remains to be determined, and improved consensus regarding the assessment of body composition is required to ascertain this.

dual-energy X-ray absorptiometry (DXA)] in patients with stage 3 or 4 nonsmall cell lung cancer, but not hand grip strength (HGS: the functional co-primary end-point) [3▪]. When the treatment period was extended for another 12 weeks in patients with a preserved Eastern Clinical Oncology Group (ECOG) status ($\leq$2), there was again a failure to demonstrate an improvement in HGS compared with placebo [4▪].

In comparison, enobosarm [a nonsteroidal selective androgen receptor modulator (GTx)] was analysed in the POWER (Prevention and treatment Of muscle Wasting in patients with cancER) 1 and 2 studies. These studies found that the use of enobosarm was also associated with an increase in LBM, but not with increased function as measured by the co-primary end-point of at least 10% improvement in stair climb power [5]. Several recent observational studies have been able to show associations between muscle mass and physical function in a range of muscle wasting conditions, including chronic kidney disease [6], elderly age [7], hip fracture [8], inflammatory myopathies [9] and sarcopenia in the context of obesity and metabolic syndrome [10]. Thus, inability of cachexia trials to demonstrate a related and synchronous increase in muscle mass and function is somewhat unexpected, and in this review, we describe the potential reasons for this observed lack of association. In particular, we will discuss both artefactual factors that relate to methods of assessment of nutritional and physical function outcome measures, and physiological factors that relate to the recruited patient population. Furthermore, we propose a new nonlinear model to express the relationship between muscle mass and patient function in cancer cachexia.

## REASON FOR THE LACK OF ASSOCIATION BETWEEN MUSCLE MASS AND FUNCTION IN CANCER CACHEXIA

### Factors in the assessment of body composition and nutritional outcome measures

Imaging techniques to assess body composition and reduced muscle volumes have been used successfully for the last 30–40 years, and include computed tomography (CT) [11], MRI [12,13], ultrasound and DXA [14]. Age-related loss of muscle (termed 'sarcopenia') was originally defined diagnostically by Baumgartner *et al.* [14] as being below 2 standard deviations of the mean appendicular muscle mass of a group of healthy individuals undergoing DXA scanning. With the standard use of CT for clinical diagnostic purposes in cancer, CT body composition analysis has emerged widely as a new potential gold standard to identify sarcopenia in cancer patients. Moreover, CT cut points for sarcopenia are described for diagnostic purposes using the consensus definition of cancer cachexia [15]. In the future, it seems likely that CT body composition analysis will be used as both an inclusion criterion and an outcome measure in RCTs for cachexia. However, the exact methodology to utilize is still unclear, and the final chosen technique will influence the observed relationship with physical function.

### Computed tomography skeletal muscle index

Recognizing the increasing use of CT in cancer staging, Mourtzakis *et al.* [11] utilized a cohort of patients undergoing both DXA and CT to produce an equation converting the original DXA measures of LBM to those of muscle cross-sectional area produced by CT at the lumbar L3 level. Such area measurements can be indexed for height to derive estimates of whole-body muscularity (skeletal muscle index: SMI), which in turn have been shown to be of prognostic value in terms of patient survival and postoperative morbidity [16,17▪]. Noting the increasing prevalence of obesity in the general population [18], Martin *et al.* [19] used a cohort of lung and gastrointestinal cancer patients to advance the use of sarcopenic diagnostic cut-points by assessing the influence of patient sex and whether or not they had high or low BMI. A method of relating the original research by Baumgartner *et al.* to the current

cancer patient phenotype seen in modern practice is of paramount clinical importance. However, despite such efforts to validate and standardize L3-CT diagnosis of sarcopenia, authors continue to use different measures (e.g. psoas muscle area) and different cutpoints [20▪▪,21,22▪], with no agreed consensus. Although frustrating, such a varied approach is understandable when one considers the various populations that have been studied. The cut-points derived by Martin *et al.* [19] were from a Canadian cohort, which from the Alberta census consists of a 70% European origin population [23]. It is known that for DXA scanning, cut-points for defining sarcopenia are different in European populations and Asian populations. Specifically, the EWGSOP (European Working Group on Sarcopenia in Older People) [24] defines low muscle mass in European females below 5.5 $kg/m^2$, whereas in a Korean population, Kwon *et al.* [21] found the cut-point to be 4.4 $kg/m^2$. Similarly, for CT-derived variables, Fujiwara *et al.* [25] found different cut-points in a Japanese cohort to those developed in the Canadian group. Some studies have shown that it is sarcopenic obesity that carries the worst clinical risk [26], whereas more recently, the negative prognostic impact of subcutaneous fat [27,28] has been demonstrated. Indeed, we have previously identified a high prevalence of sarcopenia in healthy controls when using cut points derived in a cancer population [29]. It, therefore, seems that population-specific cut-points for SMI need to be derived in any trial population before assessing muscle function.

### Computed tomography skeletal muscle radiodensity

An alternative way to assess muscle nutritional response to pharmacological intervention by CT would be to assess muscle quality rather than quantity, by measuring the radio-density of skeletal muscle in Hounsfield Units. The effect of low skeletal muscle radiodensity (SMD) on outcome has been noted in several diseases. In pancreatitis, Grinsven *et al.* [30] found a decrease in muscle radio-density during the course of the disease resulted in poorer survival. Similar findings were noted in pancreatic cancer by van Dijk *et al.* [31▪▪] where in a prospective study involving 199 patients with head of pancreas cancer, medium or low muscle radio-density was associated with worsened survival. Poorer survival was also noted in patients with endometrial cancer studied by Paula *et al.* [32], and in patients with palliative pancreatic cancer studied by Rollins *et al.* [17▪]. In these cohorts, the combination of low muscle mass with low SMD appeared to worsen outcome. In the latter study, previously published muscle mass and density cut-points [19] were used to stratify patients into sarcopenic/normal and myosteatotic/normal. Both sarcopenia and myosteatosis carried poorer prognosis, but in combination these factors were synergistic in their survival reduction. However, there is some preliminary evidence that SMD may correlate more closely with physical function in elderly cancer patients compared with SMI [33].

The inference, in line with the observations of Martin *et al.* [19], is that low SMD is the result of altered muscle composition at a cellular level, and that protein is replaced by intramuscular fat. However, once again, this assumption may not represent the entire picture. We have previously demonstrated a positive, but only poor, correlation between SMD and rectus abdominis muscle protein content in an upper gastrointestinal cancer cohort [34▪], suggesting that any change in CT variables may not necessarily confer an equivalent change in muscle composition.

Similar to SMI, it seems that radio-density cut-points also require specific development for each population under study. Studies performed by differing centres have found differing radio-density cut-points apply to their cohorts [31▪▪,35], and a recent review noted similar findings [36] throughout the literature.

Increasing interest in muscle radio-density has led to concerns regarding the effect of intravenous CT contrast media on body composition variables derived from scans performed in different phases. Earlier authors did not disclose which CT scan phases were used in their manuscripts [11,19]. However, work carried out by van Vugt *et al.* [37▪▪] investigated 50 liver transplant patients undergoing triple-phase CT scans and found statistically and clinically significant differences in SMD between noncontrast and contrast-enhanced scan phases, but not in muscle area. Applying the Martin *et al.* [19] criteria to these values resulted in 80% of patients having low SMD in unenhanced scan phases whilst 50 and 38% had low SMD in arterial and portal venous phases, respectively. In a population of pancreatic cancer patients, Rollins *et al.* found that there was a statistically significant difference between contrast phases on CT; however, there was a linear relationship between these phases, which could be translated via simple equations to provide comparable values [23]. In more closely defining the relationship between contrast phases, these authors have moved the literature forward to allow abdominal CT scans performed for any clinical indication to be compared. However, such phase-dependent values will obviously impact on any statistical relationship with physical function outcome measures. This problem can be compounded further by other CT technique-dependent

variables such as tube potential [38]. Authors are now turning to complex nonlinear trimodal regression analysis techniques of entire radiodensitometric muscle distributions to compare with standard CT metrics and lower limb muscle function [39].

## Factors in the assessment of physical function outcome measures

The identity of the best physical function outcome measure to relate nutritional outcome measures, including CT, is unknown. There is a lack of physical function tests that specifically analyse upper abdominal/L3 muscle activity. Equally, it is an unproven assumption that L3-CT should, in some way, correlate with either targeted assessments of isolated limb strength and power, or complex whole-body assessments of physical function [e.g. Timed-Up and Go-Test (TUG)] [40]. For example, we have shown previously that cancer cachexia defined using CT-derived sarcopenic cut-points can be identified in patients with normal TUG times [41].

We have previously advocated physical activity meters as devices to measure global patient function in the free-living environment [42,43]. The advantage of such devices is that they offer multiple outcome measures from a single application. However, once again, for the purposes of clinical trial design, a single outcome measure is required to be prechosen as co-primary endpoint. HGS has been used widely in nutritional studies in this role, and has been validated in various populations [44,45]. However, as described previously, it was unsuccessful in the ROMANA studies. From a clinical perspective, it might seem that measures of upper limb physical function might be less meaningful or less affected by pharmacological intervention when compared with measures of lower limb physical function, which in turn might dictate activities of daily living and overall exercise limitation. However, stair climb power was unchanged in the POWER studies [5].

## Patient factors in the trial population

An alternative explanation for the failure of previously trialled interventions to achieve significant improvements in functional end-points is an error in understanding of the nature of the relationship between muscle mass and function. To date, trial design has assumed a strong and linear relationship between muscle mass and function. However, it seems likely that, the relationship will alter dependent on the patient's morbid status at the time. The prolonged immobility, reduced food intake, and muscle depletion of a patient with refractory cachexia are likely to accelerate the rate of functional decline compared with a patient with precachexia. Equally, a patient with precachexia/cachexia may be more responsive or demonstrate faster functional improvements after weight/muscle gain compared with a patient with refractory cachexia, who may be functionally bedbound. Therefore, rather than a linear relationship between muscle mass and physical function, we propose a more complex sigmoid relationship that pursues a delayed path on recovery following initial decline (see Fig. 1). We suggest that in the early stages of disease, functionality is maintained despite loss of muscle mass, and that outcome measures of functionality currently in use are insufficiently sensitive to detect the subtle changes at this stage. These precachectic patients are likely to have a minimal functional effect from increasing muscle mass but would be most likely to benefit from prophylactic intervention to prevent further muscle mass loss and subsequent functional decline. Secondly, after a significant amount of muscle mass has been lost, function begins to decline at an increased rate. It is these cachectic patients who, we suggest, are likely to have the greatest maximal effect from interventions to preserve muscle mass. Thirdly, once a critical mass of muscle has been lost, function declines rapidly and likely in an irreversible fashion, resulting in refractory cachexia. Interventions at this stage to improve muscle mass and function are unlikely to be successful because of the refractory nature of the patient's condition, and a palliative approach may be more appropriate. The high rates of patient attrition in previous cancer cachexia trials means that is highly likely that they have recruited significant numbers of such patients. In recruiting for future trials, it would be important to recognize these patients as a distinct sub-group in whom mass and functional improvements may be futile. Fourthly, postintervention, we suggest that the recovery of muscle mass long precedes functional recovery. This is frequently seen in studies of patients recovering from critical illness. Finally, we suggest that the curve described by patients on the cachexia pathway can be shifted in a deleterious direction by the presence of systemic inflammation, comorbidities, or advanced age as frequently reported in the literature and seen clinically. This hypothetical model requires evidence in the form of larger observational studies, in combination with data from current clinical trials. Furthermore, it is highly likely that if proven, such a model will require adjustment for patient age [46] and sex [47], with transient factors such as sleep, depression and fatigue [48], as well as menopausal and habitual exercise status [49]. For example, we have shown previously that lower limb muscle strength and power assessments exhibit sexual dimorphism in patients with cancer cachexia

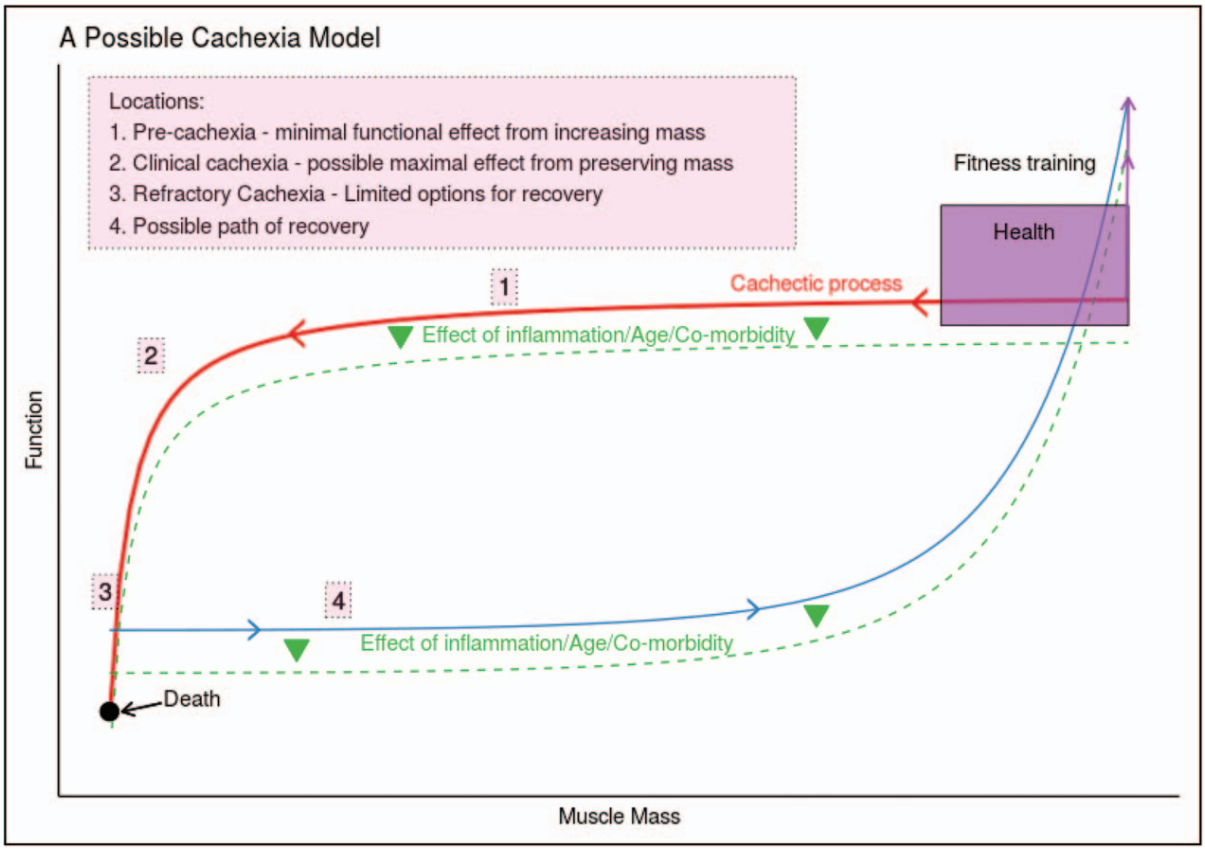



**FIGURE 1.** Proposed relationship between muscle mass and function.

[50]. However, the aim is that by carrying out model validation work, target groups for intervention trials can be identified accurately and the appropriate outcome measures more easily chosen.

## CONCLUSION

Imaging methodology and physical function investigation have advanced greatly over the last 30–40 years. Increasing numbers of variables can, thus, be interrogated and related to patient outcomes; however, the absence of a consensus on interpreting and reporting these outcomes, and the methods used to collect them, has led to variability in practice and conflicting findings. The true relationship between muscle mass and function remains to be determined, and is undoubtedly influenced by both variability in reporting practice and patient factors. Larger modeling studies based on best practice methodology are required to ascertain accurately the relationship between muscle mass and function at all timepoints on the cachectic patient's journey.

### Acknowledgements

*We would like to acknowledge the late Professor Fearon for his guidance and tutelage in this subject over many years.*

### Financial support and sponsorship

*M.I.R. received a grant from the Royal College of Surgeons of Edinburgh in 2015. M.I.R.'s salary was supported through the University of Edinburgh by Novartis in 2015/2016 and by ESPEN in 2016/2017. R.J.E.S. holds the post of NHS Research Scotland (NRS) Clinician.*

### Conflicts of interest

*M.I.R.'s salary was supported previously by Novartis. The authors declare no other conflicts of interest.*

## REFERENCES AND RECOMMENDED READING

Papers of particular interest, published within the annual period of review, have been highlighted as:
- ■ of special interest
- ■■ of outstanding interest

1. Fearon K, Argiles J, Baracos V, *et al.* Request for regulatory guidance for cancer cachexia intervention trials. J Cachexia Sarcopenia Muscle 2015; 6:272–274.
2. Knechtle B, Rüst CA, Knechtle P, Rosemann T. Does muscle mass affect running times in male long-distance master runners? Asian J Sports Med 2012; 3:247–256.
3. ■ Temel JS, Abernethy AP, Currow DC, *et al.* Anamorelin in patients with nonsmall-cell lung cancer and cachexia (ROMANA 1 and ROMANA 2): results from two randomised, double-blind, phase 3 trials. Lancet Oncol 2016; 17:519–531.

This article delivers and discusses the results of one of the most recent large cachexia studies performed, and shows increase in muscle mass without corresponding increase in function.

4. Currow D, Temel JS, Abernethy A, *et al.* ROMANA 3: a phase 3 safety ■ extension study of anamorelin in advanced nonsmall-cell lung cancer (NSCLC) patients with cachexia. Ann Oncol Off J Eur Soc Med Oncol 2017; 28:1949–1956.

This article discusses the extended results of the Anamorelin trial, and again shows increase in muscle mass without corresponding increase in function.

5. Crawford J, Johnston MA, Taylor RP, *et al.* Enobosarm and lean body mass in patients with nonsmall cell lung cancer. J Clin Oncol 2014; 32(15 Suppl):9618.
6. Highton PJ, Neale J, Wilkinson TJ, *et al.* Physical activity, immune function and inflammation in kidney patients (the PINK study): a feasibility trial protocol. BMJ Open 2017; 7:e014713.
7. Altubasi I. Physical performance of elderly adults in association with thigh tissue composition: a cross-sectional study. J Phys Ther Sci 2017; 29:2194–2198.
8. Steihaug OM, Gjesdal CG, Bogen B, *et al.* Sarcopenia in patients with hip fracture: a multicenter cross-sectional study. PLoS One 2017; 12:e0184780.
9. Cleary LC, Crofford LJ, Long D, *et al.* CT based muscle density predicts muscle function and health-related quality of life in patients with idiopathic inflammatory myopathies. Arthritis Care Res 2015; 67:1031–1040.
10. Rosique-Esteban N, Babio N, Diaz-López A, *et al.* Leisure-time physical activity at moderate and high intensity is associated with parameters of body composition, muscle strength and sarcopenia in aged adults with obesity and metabolic syndrome from the PREDIMED-Plus study. Clin Nutr 2018. [Epub ahead of print]
11. Mourtzakis M, Prado CMM, Lieffers JR, *et al.* A practical and precise approach to quantification of body composition in cancer patients using computed tomography images acquired during routine care. Appl Physiol Nutr Metab 2008; 33:997–1006.
12. Mersmann F, Bohm S, Schroll A, *et al.* Muscle shape consistency and muscle volume prediction of thigh muscles. Scand J Med Sci Sports 2015; 25:e208–e213.
13. Willis TA, Hollingsworth KG, Coombs A, *et al.* Quantitative muscle MRI as an assessment tool for monitoring disease progression in LGMD2I: a multicentre longitudinal study. PLoS One 2013; 8:e70993.
14. Baumgartner RN, Koehler KM, Gallagher D, *et al.* Epidemiology of sarcopenia among the elderly in New Mexico. Am J Epidemiol 1998; 147:755–763.
15. Fearon K, Strasser F, Anker SD, *et al.* Definition and classification of cancer cachexia: an international consensus. Lancet Oncol 2011; 12:489–495.
16. Parsons HA, Baracos VE, Dhillon N, *et al.* Body composition, symptoms and survival in advanced cancer patients referred to a phase I service. PLoS One 2012; 7:e29330.
17. Rollins KE, Tewari N, Ackner A, *et al.* The impact of sarcopenia and myos- ■ teatosis on outcomes of unresectable pancreatic cancer or distal cholangiocarcinoma. Clin Nutr Edinb Scotl 2016; 35:1103–1109.

This article shows the synergistic effects of low muscle mass together with low muscle radio-attenuation in pancreatic cancer sufferers.

18. Agha M, Agha R. The rising prevalence of obesity: part A: impact on public health. Int J Surg Oncol 2017; 2:e17.
19. Martin L, Birdsell L, MacDonald N, *et al.* Cancer cachexia in the age of obesity: skeletal muscle depletion is a powerful prognostic factor, independent of body mass index. J Clin Oncol 2013; 31:1539–1547.
20. Rutten IJG, Ubachs J, Kruitwagen RFPM, *et al.* Psoas muscle area is not ■■ representative of total skeletal muscle area in the assessment of sarcopenia in ovarian cancer. J Cachexia Sarcopenia Muscle 2017; 8:630–638.

This article adds a degree of clarity in the body composition field by comparing the values obtained in whole-slice CT analysis with those obtained by psoas-alone analysis and finds that they do not closely correlate.

21. Kwon H-J, Ha Y-C, Park H-M. The reference value of skeletal muscle mass index for defining the sarcopenia of women in Korea. J Bone Metab 2015; 22:71–75.
22. Werf A, Langius JaE, Schueren MAE, *et al.* Percentiles for skeletal muscle ■ index, area and radiation attenuation based on computed tomography imaging in a healthy Caucasian population. Eur J Clin Nutr 2018; 72:288–296.

This article provides a range of data covering what could be considered to be normal values of CT body composition variables in a normal healthy population.

23. Government of Canada SC. Immigration and Ethnocultural Diversity Highlight Tables - Ethnic Origin, both sexes, age (total), Alberta, 2016 Census – 25% sample data. 2017 [cited 2018 Jun 18]. Available at: https://www12.statcan.gc.ca/census-recensement/2016/dp-pd/hlt-fst/imm/Table.cfm?Lang=E&T=31&Geo=48&SO=4D.
24. Cruz-Jentoft AJ, Baeyens JP, Bauer JM, *et al.* Sarcopenia: European consensus on definition and diagnosis. Age Ageing 2010; 39:412–423.
25. Fujiwara N, Nakagawa H, Kudo Y, *et al.* Sarcopenia, intramuscular fat deposition, and visceral adiposity independently predict the outcomes of hepatocellular carcinoma. J Hepatol 2015; 63:131–140.
26. Tan BHL, Birdsell LA, Martin L, *et al.* Sarcopenia in an overweight or obese patient is an adverse prognostic factor in pancreatic cancer. Clin Cancer Res 2009; 15:6973–6979.
27. Ebadi M, Martin L, Ghosh S, *et al.* Subcutaneous adiposity is an independent predictor of mortality in cancer patients. Br J Cancer 2017; 117:148–155.
28. Montano-Loza AJ, Mazurak VC, Ebadi M, *et al.* Visceral adiposity increases risk for hepatocellular carcinoma in male patients with cirrhosis and recurrence after liver transplant. Hepatology 2018; 67:914–923.
29. Ramage MI, Staniszewska A, Schuepbach E, *et al.* Low muscularity by CT definition is present in both cancer and noncancer control populations. Abstracts of the 10th International Conference on Cachexia, Sarcopenia and Muscle Wasting, Rome, Italy, 8–10 December 2017 (Part 2). J Cachexia, Sarcopenia Muscle 2018; 9:183–212.
30. Grinsven F van, Vugt JLA van, Gharbharan A, *et al.* The association of computed tomography-assessed body composition with mortality in patients with necrotizing pancreatitis. J Gastrointest Surg 2017; 21: 1000–1008.
31. van Dijk DPJ, Bakens MJAM, Coolsen MME, *et al.* Low skeletal muscle ■■ radiation attenuation and visceral adiposity are associated with overall survival and surgical site infections in patients with pancreatic cancer. J Cachexia Sarcopenia Muscle 2017; 8:317–326.

This study complements the emerging literature on muscle radiodensity in cancer survival and builds upon the idea that population-specific cut-points may need to be identified and calculated for each study performed in this area.

32. Paula NS, Rodrigues CS, Chaves GV. Comparison of the prognostic value of different skeletal muscle radiodensity parameters in endometrial cancer. Eur J Clin Nutr 2018. [Epub ahead of print]
33. Williams GR, Deal AM, Muss HB, *et al.* Skeletal muscle measures and physical function in older adults with cancer: sarcopenia or myopenia? Oncotarget 2017; 8:33658–33665.
34. Ramage MI, Johns N, Deans CDA, *et al.* The relationship between muscle ■ protein content and CT-derived muscle radio-density in patients with upper GI cancer. Clin Nutr 2018; 37:752–754.

This article details a range of muscle protein content values obtained using modern techniques more readily available than those used to create reference values.

35. Antoun S, Lanoy E, Iacovelli R, *et al.* Skeletal muscle density predicts prognosis in patients with metastatic renal cell carcinoma treated with targeted therapies. Cancer 2013; 119:3377–3384.
36. Kazemi-Bajestani SMR, Mazurak VC, Baracos V. Computed tomography-defined muscle and fat wasting are associated with cancer clinical outcomes. Semin Cell Dev Biol 2016; 54:2–10.
37. van Vugt JLA, Coebergh van den Braak RRJ, Schippers HJW, *et al.* Contrast- ■■ enhancement influences skeletal muscle density, but not skeletal muscle mass, measurements on computed tomography. Clin Nutr 2017. [Epub ahead of print]

This study investigates the effect of confounding factors in the field of CT body composition analysis with specific regards to CT contrast phase, adding certainty to previously raised concerns in this area and calling for clarity in reporting.

38. Morsbach F, Zhang Y-H, Nowik P, *et al.* Influence of tube potential on CT body composition analysis. Nutrition 2018; 53:9–13.
39. Edmunds K, Gíslason M, Sigurðsson S, *et al.* Advanced quantitative methods in correlating sarcopenic muscle degeneration with lower extremity function biometrics and comorbidities. PLoS One 2018; 13:e0193241.
40. Macdonald AJ, Stephens N, Greig CA, *et al.* PP083-SUN CT-derived measures of muscle mass are associated with functional ability but not strength and power in patients with upper GI cancer. Clin Nutr 2013; 32:S53.
41. Ramage MI, Miller J, Schuepbach E, *et al.* The emerging disconnect between muscle mass and function: evidence from different patient populations. Abstracts of the 10th International Conference on Cachexia, Sarcopenia and Muscle Wasting, Rome, Italy, 8–10 December 2017 (Part 2). J Cachexia, Sarcopenia Muscle 2018; 9:202.
42. Skipworth RJE, Stene GB, Dahele M, *et al.* Patient-focused endpoints in advanced cancer: criterion-based validation of accelerometer-based activity monitoring. Clin Nutr 2011; 30:812–821.
43. Ferrioli E, Skipworth RJE, Stenseth J, *et al.* Abstract 142; Abstracts of the 5th Cachexia Conference, Barcelona, Spain, December 5–8, 2009. J Cachexia Sarcopenia Muscle 2010; 1:43–128.
44. Lee SH, Kim SJ, Han Y, *et al.* Hand grip strength and chronic obstructive pulmonary disease in Korea: an analysis in KNHANES VI. Int J Chron Obstruct Pulmon Dis 2017; 12:2313–2321.
45. Norman K, Stobäus N, Smoliner C, *et al.* Determinants of hand grip strength, knee extension strength and functional status in cancer patients. Clin Nutr 2010; 29:586–591.
46. Perkin OJ, McGuigan PM, Thompson D, Stokes KA. Habitual physical activity levels do not predict leg strength and power in healthy, active older adults. PLoS One 2018; 13:e0200089.
47. Resnick B, Hebel JR, Gruber-Baldini AL, *et al.* The impact of body composition, pain and resilience on physical activity, physical function and physical performance at 2 months post hip fracture. Arch Gerontol Geriatr 2018; 76:34–40.
48. Brady AO, Straight CR. Muscle capacity and physical function in older women: what are the impacts of resistance training? J Sport Health Sci 2014; 3:179–188.
49. Straight CR, Ward-Ritacco CL, Evans EM. Association between accelerometer-measured physical activity and muscle capacity in middle-aged postmenopausal women. Menopause 2015; 22:1204–1211.
50. Stephens NA, Gray C, MacDonald AJ, *et al.* Sexual dimorphism modulates the impact of cancer cachexia on lower limb muscle mass and function. Clin Nutr 2012; 31:499–505.

## Appendix C

# Patient Information and Consent Forms

# Biochemical and functional biomarkers of cachexia: Information for non-cancer patients

You are being invited to take part in a research study. Before you decide, it is important for you to understand why the research is being done and what it will involve. Please take time to read the following information carefully. Talk to others about the study if you wish.

- Part 1 tells you the purpose of this study and what will happen to you if you take part.
- Part 2 gives you more detailed information about the conduct of the study.

Ask us if there is anything that is not clear or if you would like more information. Take time to decide whether or not you wish to take part.

## Part 1

### What is the purpose of the study?

The purpose of this research study is to find out what causes people who have cancer to suffer muscle wasting, lose weight and feel tired. This wasting is a significant problem for patients as it causes a reduction in both quality and quantity of life. In order to find out what causes this problem and if there is any way to predict its development, we are asking patients about to undergo surgery for cancer if they would undergo a variety of tests before, during, and after their operation. These tests are mainly to measure changes in the size and function of the patients' muscle and fat, and to see whether these changes can be predicted. In particular, we would like to take small samples of muscle, tumour, fat, blood, and urine during the patients' operation to see if there are any biochemical changes. We aim to follow up the patients by asking for more blood and urine, as well as a needle test of the thigh muscle at clinic appointments up to 12 months after the operation. These tests are designed to cause the minimum of discomfort or inconvenience for anyone involved.

In order to find out whether the changes we see in these tests are purely related to cancer, we are also asking patients who do not have cancer, but are having surgery for other reasons, to join the study as a control group for comparison. The control group will take part in the some, but not all, of the tests undertaken by the cancer patients.

**Why have I been invited?**

You have been invited because you will soon be having surgery unrelated to cancer. You will be one of the healthy controls. We aim to recruit 20 participants in total. Patients undergoing purely keyhole (laparoscopic) surgery are not being invited for technical reasons.

**Do I have to take part?**

No. It is up to you to decide whether or not to take part. If you do, you will be given this information sheet to keep and be asked to sign a consent form by one of the research team. You will still be free to withdraw at any time in the future and without giving a reason. A decision to withdraw at any time, or a decision not to take part, will not affect the standard of care that you receive.

**What will happen to me if I take part?**

If you take part, you will be asked to undergo certain tests before your operation, during your operation, and at 2-3 appointments after the operation. The study appointments before and after your operation will be timed to coincide with your normal clinic appointments. Therefore, no extra visits to hospital are required if you choose to take part in this study. We anticipate the research visits will add no more than 15 minutes to your appointment. However, if for any reason we are unable to coincide your study appointments with your normal clinic appointments, we will provide taxi transport to and from the Royal Infirmary of Edinburgh's Clinical Research Facility. The tests will include:

- ***A blood test:*** The amount of blood taken is approximately the same as one teaspoonful (5ml), and it will be taken from your arm in the usual fashion.

- ***A urine test:*** This will be performed in the usual fashion. The amount of urine taken is 20-30ml.

- ***Tests of normal activity:*** We would like to see how much activity you do during a normal week. To help assess this, we may ask you to wear a small, light physical activity meter on your thigh, under a waterproof dressing, for a week at a time. This does not inconvenience most patients, and they quickly forget that they are wearing it. These monitors can be returned by post in a pre-paid envelope you will be given.

- ***Test of functional ability (timed up-and-go and 6 minute walk):*** This test will measure your ability to get up out of a chair and walk a few metres then turn around and go back to your chair. It will only take a few minutes. The 6 minute walk test measures how far you can walk in 6 minutes. This will be done over a measured course, and should not be tiring. It can be stopped at any time if necessary.

- ***A questionnaire:*** This will take approximately 15 minutes to complete but can be taken home to do. We can provide a stamped-addressed envelope to post it to us if you take it home.

- ***CT scan review:*** We also ask that you give us permission to review the CT scans that you will have had performed as part of your routine care. This review is simply to assess the amounts of muscle and fat in your body, and does not influence your treatment in any way. The review will be performed by a member of the research team.

We would initially plan to carry out all of the above tests at one appointment prior to the date of your operation. If, after carrying them out, you found that

these tests were acceptable to perform (as we would hope), we would ask you to repeat the same tests, including the blood and urine samples, at the usual follow-up clinic appointments after your surgery for a period of up to 12 months.

If at one of the appointments after your operation you are still willing to participate, we would ask that you give us permission to take a thigh muscle biopsy at that time. This will be performed under local anaesthetic injection but will otherwise be the same as the one that you had during your operation. This will involve a local anaesthetic injection, and will leave a small scar of 2-3 millimetres (which will be closed with paper stitches). This biopsy is usually well-tolerated, but may leave you with some discomfort for a day or so, and a small bruise. We will re-confirm your permission to perform this test at that time.

In addition to the tests described above, we will also ask permission to take tissue samples during your operation, whilst you are asleep (under general anaesthetic):

- ***A muscle biopsy:*** Whilst you are asleep (under general anaesthetic) during your operation, small muscle samples will be removed from both your abdominal wall and your thigh. The abdominal muscle sample will be pea-sized and will be taken from the incision through which your operation is being performed. This will not complicate your operation. The amount of muscle removed from the front of your thigh is even smaller and is taken via a tiny incision (a few millimeters long) through which a needle is introduced. This incision will be closed by paper stitches and will leave a small mark which will fade. If you consent to have a thigh muscle biopsy in the outpatient clinic, this will be done under local anaesthetic.

- ***A fat biopsy*****:** When you are asleep under general anaesthetic, a small piece of fat (again, about the size of a pea) will be taken from just under the skin and also from the fat layer inside of your abdomen.

- ***Blood and urine tests:*** If not taken in clinic before your operation, these could be taken whilst asleep under general anaesthetic.

**What do I have to do?**

Apart from attendance at the appointments before your operation, and after you operation, no other responsibilities are required from your participation. We would like your permission to monitor your case for up to 5 years after your operation to see how things are going.

**What are the possible disadvantages and risks of taking part?**

We have taken every step in the design of this study to minimise any possible disadvantages and risks.

The tissue samples taken during your operation may cause a slightly increased risk of bleeding. This risk is very small, and the nature of the samples, together with the samples being taken by the operating surgeon, means the risk is very small indeed.

Regarding the thigh muscle biopsy, you may experience some mild discomfort and stiffness in the leg following the procedure, but this should wear off after a few hours. Also, approximately 1 in 200 people suffer with bruising following the procedure. This has been described as similar to a “dead leg” but fades in 1-2 days.

If you have are having a repeat thigh muscle biopsy this will be performed under a local anaesthetic injection. The anaesthetic agent may sting for a few seconds as it is being administered.

If you have any problems after the needle test, please contact the research team directly using the number at the end of this sheet.

**What are the possible benefits of taking part?**

We cannot promise that the study will help you directly, but the information we receive might help improve the treatment of patients with cancer and cancer-associated weight loss.

**What happens when the research study stops?**

Following your appointment after your operation, no further appointments are required. However, if any of your blood, muscle, or urine samples remain, we would ask your permission to store these samples (in anonymised form) in the University of Edinburgh so that we can consider them for use in future research studies that we may carry out (if a local Ethics Committee deems the studies appropriate).

**Part 2**

**What if relevant new information becomes available?**

If any new treatments for your medical conditions become available during the time of the study, they will not be withheld from you because of your participation in this study, should you need them. Furthermore, if you require any treatment during the course of the study, it will not be withheld from you because of your participation in this study.

**What will happen if I don't want to carry on with the study?**

You can withdraw from the study at any time. However, we would ask your permission to keep in contact with you to monitor your progress. In this way, any information that was collected during the time of your participation in the study may still be used for research purposes. Any stored blood or tissue samples that can still be identified as yours will be destroyed if you wish.

If for any reason you become unable to make decisions regarding your health, we would stop collecting information and samples from you, and we would not invite you to any more appointments. We would ask to keep and use any samples we had already collected.

**What if there is a problem?**

- **Complaints:** If you have a concern about any aspect of this study, you should ask to speak with the researchers who will do their best to answer your question. If you remain unhappy and wish to complain formally, you can do this through the NHS Complaints Procedure. Details can be obtained from the hospital.

If you wish to make a complaint about the study, please contact NHS Lothian:

*NHS Lothian Complaints Team*
*2nd Floor*
*Waverly Gate*
*2-4 Waterloo Place*
*Edinburgh*
*EH1 3EG*
*Tel: 0131 465 5708*
*Email: craft@nhslothian.scot.nhs.uk*

- **Harm:** In the unlikely event that something goes wrong and you are harmed during the research and this is due to someone's negligence then you may have grounds for a legal action for compensation against NHS Lothian but you may have to pay your legal costs. The normal National Health Service complaints mechanisms will still be available to you (if appropriate).

**Will my taking part in this study be kept confidential?**

Yes. All information which is collected about you during the course of the research will be kept strictly confidential. However, we would like to inform your GP of your involvement in this study but we will require your permission to do this. All other information about you which leaves the Royal Infirmary of Edinburgh will have your name and address removed so that you cannot be recognised from it.

If any information comes to light during the study which may have a bearing on your care, we would aim to inform the team looking after you, and with your consent would also inform your GP.

Muscle, blood, fat and urine samples collected during the study may be transferred for the purpose of analysis to associated researchers within and outside the European Economic Area, including to commercial companies such as Novartis (a pharmaceutical company). All samples will be anonymised prior

to sending and therefore you will not be identifiable. However, there will be a link from your samples to your records so we can match any changes in your case (or your withdrawal) to your samples.

**What will happen to any samples I give?**

A portion of the blood samples will be immediately analysed by the Department of Biochemistry at the Royal Infirmary of Edinburgh. The remainder of the blood samples, along with the muscle, fat, and urine samples, will be transferred to the University of Edinburgh for analysis. The only individuals who will have direct access to these samples will be the members of the research team behind this study. The samples will be analysed in the University of Edinburgh by various biochemical techniques in order to measure the levels of certain 'markers' of wasting within the various tissues.

**To make sure all information remains confidential, all personal identifiers on your sample will be removed and replaced with a code. Your personal and medical history data will be stored separately and will not be processed. The key that links your data to the code on the sample will be stored in a secure location to which only authorized personnel of the SCHOOL of CLINICAL SCIENCES will have access. This is a "linked-anonymised" system. Your coded samples may also be transferred for analysis to third parties in or outside the UK. This can include collaborating academic institutions or pharmaceutical companies, which contribute to this research project.** #

#

Following all of these different analyses, if any of the samples remain, we would ask your permission to store these samples long-term (in anonymised form) in the University of Edinburgh so that we can consider them for future research studies (if a local Ethics Committee deems the studies appropriate). Professor Kenneth Fearon, Professor of Surgical Oncology, will act as custodian for any stored samples. The only other individuals who will have direct access to the stored samples will be the members of the research team behind the current study. We would aim to store the samples until they are used.

**Will any genetic tests be done on the samples that I give?**

We have no plans to perform genetic analysis within the remit of this current study. However, following this current study, we would ask your permission to store any remaining samples so that we may consider them for use in future research studies that we may carry out (if a local Ethics Committee deems the study appropriate). Future studies could potentially involve genetic analysis, but such studies are at a very early stage of planning and not yet in progress. Any results from future genetic studies will not have any healthcare implications for you and hence we would not normally feed these results back to you.

**What will happen to the results of the current research study?**

The results of this study will be published in medical journals, reports and textbooks. Results will be made available to study participants through the Cancer Research UK website. You will not be identifiable in any report/publication or report unless you have specifically consented to release such information.

**Who is organising and funding the research?**

The research is being organised and sponsored by the University of Edinburgh. The research is being funded by Novartis.

**Who has reviewed the study?**

This study was given a favourable ethical opinion for conduct in the NHS by the South East Scotland Research Ethics Committee. NHS Lothian Management Approval has been obtained, and this study has also been reviewed by members of the scientific committee of Novartis.

**Contact details**

You may contact me (the main researcher) directly by telephoning 0131 242 6520 for further information at any time. Alternatively, if you wish to discuss this research study with someone independent of the research team, you can

contact Professor Wigmore in the Department of Surgery, who is acting as an independent advisor – contact 0131 242 3615.

**Many thanks for your time.**

**Mr. Michael Ramage**
**Clinical Research Fellow**
**Department of Surgery**
**Royal Infirmary of Edinburgh**

Participant Consent Form Version 2 - 15 Oct 2015

Patient details

# Biochemical and functional biomarkers of cachexia in cancer patients

Please initial

1. I agree to take part in the above-titled study. ☐

2. I confirm that I have read and understand the information sheet **version 2 dated 15/10/2015** for the above study. I have had the opportunity to consider the information and ask questions, and I have had these answered satisfactorily. ☐

3. I understand that my participation is voluntary and that I am free to withdraw at any time, without giving any reason, without my medical care or legal rights being affected. ☐

4. I understand that relevant sections of any of my medical notes and data collected during the study may be looked at by individuals from the Sponsor [University of Edinburgh and NHS Lothian], from the NHS organisation or other authorities, where it is relevant to my taking part in this research. I give permission for these individuals to have access to my records. ☐

5. I agree to my GP being informed of my participation in the study. ☐

6. I agree to my GP being informed of any information found during the study which may have a bearing on my care. ☐

7. I agree to the storage of anonymised samples taken during the course of this study so that they may be considered for use in future research studies (pending a favourable ethical opinion by Lothian Local Research Ethics Committee). ☐

8. I agree to the use of samples taken during the course of this study in possible future genetic studies (pending a favourable ethical opinion by Lothian Local Research Ethics Committee). *(Optional. Only initial if you agree)* ☐

9. I understand that my anonymised samples and anonymised data may be distributed to third parties such as academic institutions or pharmaceutical companies in or outside of the UK who participate in the project ☐

10. I understand that the results of this study may be used for future commercial development of products/tests/treatments and I will not benefit financially from this ☐

| _____ | _____ | _____ |
|---|---|---|
| Name of patient | Date | Signature |
| _____ | _____ | _____ |
| Person taking consent | Date | Signature |

Original (x1) to be retained in site file. Copy (x1) to patient notes. Copy (x1) to patient

# Biochemical and functional biomarkers of cachexia: Information for cancer patients

You are being invited to take part in a research study. Before you decide, it is important for you to understand why the research is being done and what it will involve. Please take time to read the following information carefully. Talk to others about the study if you wish.

- Part 1 tells you the purpose of this study and what will happen to you if you take part.
- Part 2 gives you more detailed information about the conduct of the study.

Ask us if there is anything that is not clear or if you would like more information. Take time to decide whether or not you wish to take part.

## Part 1

### What is the purpose of the study?

The purpose of this research study is to find out what causes people who have cancer to suffer muscle wasting, lose weight and feel tired. This wasting is a significant problem for patients as it causes a reduction in both quality and quantity of life. In order to find out what causes this problem and if there is any way to predict its development, we are asking patients about to undergo surgery for cancer if they would undergo a variety of tests before, during, and after their operation. These tests are mainly to measure changes in the size and function of the patients' muscle and fat, and to see whether these changes can be predicted. In particular, we would like to take small samples of muscle, tumour, fat, blood, and urine during the patients' operation to see if there are any biochemical changes. We aim to follow up the patients by asking for more blood and urine, as

well as a needle test of the thigh muscle at clinic appointments up to 12 months after the operation. These tests are designed to cause the minimum of discomfort or inconvenience for anyone involved.

In order to find out whether the changes we see in these tests are purely related to cancer, we are also asking patients who do not have cancer, but are having surgery for other reasons, to join the study as a control group for comparison. The control group will take part in the some, but not all, of the tests undertaken by the cancer patients.

**Why have I been invited?**

You have been invited because you will soon be having surgery for cancer. We aim to recruit 100 participants in total. Patients undergoing purely keyhole (laparoscopic) surgery are not being invited for technical reasons.

**Do I have to take part?**

No. It is up to you to decide whether or not to take part. If you do, you will be given this information sheet to keep and be asked to sign a consent form by one of the research team. You will still be free to withdraw at any time in the future and without giving a reason. A decision to withdraw at any time, or a decision not to take part, will not affect the standard of care that you receive.

**What will happen to me if I take part?**

If you take part, you will be asked to undergo certain tests before your operation, during your operation, and at 2-3 appointments after the operation, for up to 12 months. The study appointments before and after your operation will be timed to coincide with your normal clinic appointments. Therefore, no extra visits to hospital are required if you choose to take part in this study. We anticipate the research visits will add no more than 15 minutes to your appointment. However, if for any reason we are unable to coincide your study appointments with your normal clinic

appointments, we will provide taxi transport to and from the Royal Infirmary of Edinburgh's Clinical Research Facility. The tests will include:

- ***A blood test:*** The amount of blood taken is approximately the same as one teaspoonful (5ml), and it will be taken from your arm in the usual fashion.

- ***A urine test:*** This will be performed in the usual fashion. The amount of urine taken is 20-30ml.

- ***Tests of normal activity:*** We would like to see how much activity you do during a normal week. To help assess this, we may ask you to wear a small, light physical activity meter on your thigh, under a waterproof dressing, for a week at a time. This does not inconvenience most patients, and they quickly forget that they are wearing it. These monitors can be returned by post in a pre-paid envelope you will be given.

- ***Test of functional ability (timed up-and-go and 6 minute walk):*** This test will measure your ability to get up out of a chair and walk a few metres then turn around and go back to your chair. It will only take a few minutes. The 6 minute walk test measures how far you can walk in 6 minutes. This will be done over a measured course, and should not be tiring. It can be stopped at any time if necessary.

- ***A questionnaire:*** This will take approximately 15 minutes to complete but can be taken home to do. We can provide a stamped-addressed envelope to post it to us if you take it home.

- ***CT scan review:*** We also ask that you give us permission to review the CT scans that you will have had performed as part of your routine care. This review is simply to assess the amounts of muscle and fat in your body, and does not influence your

treatment in any way. The review will be performed by a member of the research team.

We would initially plan to carry out all of the above tests at one appointment prior to the date of your operation. If, after carrying them out, you found that these tests were acceptable to perform (as we would hope), we would ask you to repeat the same tests, including the blood and urine samples, at the usual follow-up clinic appointments after your surgery for a period of up to 12 months.

If at one of the appointments after your operation you are still willing to participate, we would also ask that you give us permission to take a thigh muscle biopsy at that time. This will be performed under local anaesthetic injection but will otherwise be the same as the one that you had during your operation. This will involve a local anaesthetic injection, a small scar of 2-3 millimetres (which will be closed with paper stitches), and may leave you with a bruised feeling for a day or so. We will re-confirm your permission to perform this test at that time.

In addition to the tests described above, we will also ask permission to take tissue samples during your operation, whilst you are asleep (under general anaesthetic):

- ***A muscle biopsy:*** Whilst you are asleep (under general anaesthetic) during your operation, small muscle samples will be removed from both your abdominal wall and your thigh. The abdominal muscle sample will be pea-sized and will be taken from the incision through which your operation is being performed. This will not complicate your operation. The amount of muscle removed from the front of your thigh is even smaller and is taken via a tiny incision (a few millimeters long) through which a needle is introduced. This incision will be closed by paper stitches and will leave a small mark which will fade. If you consent to have a thigh muscle biopsy in the outpatient clinic, this will be done under local anaesthetic.

- ***A fat biopsy*:** When you are asleep under general anaesthetic, a small piece of fat (again, about the size of a pea) will be taken from just under the skin and also from the fat layer inside of your abdomen.

- ***Blood and urine tests:*** If not taken in clinic before your operation, these could be taken whilst asleep under general anaesthetic.

- ***A tumour biopsy:*** We will ask you permission for a sample of tumour to be taken from the specimen removed at your operation. This will be done after the tumour has been removed.

**What do I have to do?**

Apart from attendance at the appointments before your operation, and after you operation, no other responsibilities are required from your participation. We would like your permission to monitor your case for up to 5 years after your operation to see how things are going.

**What are the possible disadvantages and risks of taking part?**

We have taken every step in the design of this study to minimise any possible disadvantages and risks.

The tissue samples taken during your operation may cause a slightly increased risk of bleeding. This risk is very small, and the nature of the samples, together with the samples being taken by the operating surgeon, means the risk is very small indeed.

Regarding the thigh muscle biopsy, you may experience some mild discomfort and stiffness in the leg following the procedure, but this should wear off after a few hours. Also, approximately 1 in 200 people suffer with bruising following the procedure. This has been described as similar to a “dead leg” but fades in 1-2 days.

If you have are having a repeat thigh muscle biopsy this will be performed under a local anaesthetic injection. The anaesthetic agent may sting for a few seconds as it is being administered.

If you have any problems after the needle test, please contact the research team directly using the number at the end of this sheet.

**What are the possible benefits of taking part?**

We cannot promise that the study will help you directly, but the information we receive might help improve the treatment of patients with cancer and cancer-associated weight loss.

**What happens when the research study stops?**

Following your appointment after your operation, no further appointments are required. However, if any of your blood, muscle, or urine samples remain, we would ask your permission to store these samples (in anonymised form) in the University of Edinburgh so that we can consider them for use in future research studies that we may carry out (if a local Ethics Committee deems the studies appropriate).

**Part 2**

**What if relevant new information becomes available?**

If any new treatment for cancer or cancer-associated wasting becomes available during the time of the study, it will not be withheld from you because of your participation in this study should you need it. Furthermore, if you require any treatment for cancer or other conditions during the course of the study (e.g. chemotherapy or radiotherapy), it will not be withheld from you because of your participation in this study.

**What will happen if I don't want to carry on with the study?**

You can withdraw from the study at any time. However, we would ask your permission to keep in contact with you to monitor your progress. In this way, any information that was collected during the time of your participation in the study may

still be used for research purposes. Any stored blood or tissue samples that can still be identified as yours will be destroyed if you wish.

If for any reason you become unable to make decisions regarding your health, we would stop collecting information and samples from you, and we would not invite you to any more appointments. We would ask to keep and use any samples we had already collected.

**What if there is a problem?**

- **Complaints:** If you have a concern about any aspect of this study, you should ask to speak with the researchers who will do their best to answer your question. If you remain unhappy and wish to complain formally, you can do this through the NHS Complaints Procedure. Details can be obtained from the hospital.

  If you wish to make a complaint about the study, please contact NHS Lothian:

  *NHS Lothian Complaints Team*
  *2nd Floor*
  *Waverly Gate*
  *2-4 Waterloo Place*
  *Edinburgh*
  *EH1 3EG*
  *Tel: 0131 465 5708*
  *Email: craft@nhslothian.scot.nhs.uk*

- **Harm:** In the unlikely event that something goes wrong and you are harmed during the research and this is due to someone's negligence then you may have grounds for a legal action for compensation against NHS Lothian but

you may have to pay your legal costs. The normal National Health Service complaints mechanisms will still be available to you (if appropriate).

**Will my taking part in this study be kept confidential?**

Yes. All information which is collected about you during the course of the research will be kept strictly confidential. However, we would like to inform your GP of your involvement in this study but we will require your permission to do this. All other information about you which leaves the Royal Infirmary of Edinburgh will have your name and address removed so that you cannot be recognised from it.

If any information comes to light during the study which may have a bearing on your care, we would aim to inform the team looking after you, and with your consent we would also inform your GP.

Muscle, blood, fat and urine samples collected during the study may be transferred for the purpose of analysis to associated researchers within and outside the European Economic Area, including to commercial companies such as Novartis (a pharmaceutical company). All samples will be anonymised prior to sending and therefore you will not be identifiable. However, there will be an available link from your samples to your records so we can match any changes in your case (or your withdrawal) to your samples.

**What will happen to any samples I give?**

A portion of the blood samples will be immediately analysed by the Department of Biochemistry at the Royal Infirmary of Edinburgh. The remainder of the blood samples, along with the muscle, fat, and urine samples, will be transferred to the University of Edinburgh for analysis. The only individuals who will have direct access to these samples will be the members of the research team behind this study. The samples will be analysed in the University of Edinburgh by various biochemical techniques in order to measure the levels of certain ‘markers’ of wasting within the various tissues.

**To make sure all information remains confidential, all personal identifiers on your sample will be removed and replaced with a code. Your personal and**

**medical history data will be stored separately and will not be processed. The key that links your data to the code on the sample will be stored in a secure location to which only authorized personnel of the SCHOOL of CLINICAL SCIENCES will have access. This is a "linked-anonymised" system. Your coded samples may also be transferred for analysis to third parties in or outside the UK. This can include collaborating academic institutions or pharmaceutical companies, which contribute to this research project.** #

#

Following all of these different analyses, if any of the samples remain, we would ask your permission to store these samples long-term (in anonymised form) in the University of Edinburgh so that we can consider them for future research studies (if a local Ethics Committee deems the studies appropriate). Professor Kenneth Fearon, Professor of Surgical Oncology, will act as custodian for any stored samples. The only other individuals who will have direct access to the stored samples will be the members of the research team behind the current study.

**Will any genetic tests be done on the samples that I give?**

We have no plans to perform genetic analysis within the remit of this current study. However, following this current study, we would ask your permission to store any remaining samples so that we may consider them for use in future research studies that we may carry out (if a local Ethics Committee deems the study appropriate). Future studies could potentially involve genetic analysis, but such studies are at a very early stage of planning and not yet in progress. Any results from future genetic studies will not have any healthcare implications for you and hence we would not normally feed these results back to you.

**What will happen to the results of the current research study?**

The results of this study will be published in medical journals, reports and textbooks. Results will be made available to study participants through the Cancer Research UK website. You will not be identifiable in any report/publication or report unless you have specifically consented to release such information.

**Who is organising and funding the research?**

The research is being organised and sponsored by the University of Edinburgh. The research is being funded by Novartis.

**Who has reviewed the study?**

This study was given a favourable ethical opinion for conduct in the NHS by the South East Scotland Research Ethics Committee. NHS Lothian Management Approval has been obtained, and this study has also been reviewed by members of the scientific committee of Novartis.

**Contact details**

You may contact me (the main researcher) directly by telephoning 0131 242 6520 for further information at any time. Alternatively, if you wish to discuss this research study with someone independent of the research team, you can contact Professor Wigmore in the Department of Surgery, who is acting as an independent advisor – contact 0131 242 3615.

**Many thanks for your time.**

**Mr. Michael Ramage**
**Clinical Research Fellow**
**Department of Surgery**
**Royal Infirmary of Edinburgh**

Participant Consent Form Version 2 - 15 Oct 2015

Patient details

# Biochemical and functional biomarkers of cachexia in cancer patients

Please initial

1. I agree to take part in the above-titled study. ☐

2. I confirm that I have read and understand the information sheet **version 2 dated 15/10/2015** for the above study. I have had the opportunity to consider the information and ask questions, and I have had these answered satisfactorily. ☐

3. I understand that my participation is voluntary and that I am free to withdraw at any time, without giving any reason, without my medical care or legal rights being affected. ☐

4. I understand that relevant sections of any of my medical notes and data collected during the study may be looked at by individuals from the Sponsor [University of Edinburgh and NHS Lothian], from the NHS organisation or other authorities, where it is relevant to my taking part in this research. I give permission for these individuals to have access to my records. ☐

5. I agree to my GP being informed of my participation in the study. ☐

6. I agree to my GP being informed of any information found during the study which may have a bearing on my care ☐

7. I agree to the storage of anonymised samples taken during the course of this study so that they may be considered for use in future research studies (pending a favourable ethical opinion by Lothian Local Research Ethics Committee). ☐

8. I agree to the use of samples taken during the course of this study in possible future genetic studies (pending a favourable ethical opinion by Lothian Local Research Ethics Committee). *(Optional. Only initial if you agree)* ☐

9. I understand that my anonymised samples and anonymised data may be distributed to third parties such as academic institutions or pharmaceutical companies in or outside of the UK who participate in the project ☐

10. I understand that the results of this study may be used for future commercial development of products/tests/treatments and I will not benefit financially from this ☐

| _____ | _____ | _____ |
|---|---|---|
| Name of patient | Date | Signature |
| _____ | _____ | _____ |
| Person taking consent | Date | Signature |

Original (x1) to be retained in site file. Copy (x1) to patient notes. Copy (x1) to patient

# Appendix D

# Questionnaires

PATIENT ID

Date of Consent _____ NorSto ___

Date of Operation _____ NorOes ___

TRI0159 No _____ Tum ___

OCCAMS Y/N _____ EOTB Y/N _____

**Questionnaires** QLQ-C30 Y/N EQ-5D Y/N REFS / PG-SGA Y/N

**Exposures** Smoking – Current/Never/Ex-smoker Quit _____ Alcohol – U/Wk ☐

Cigarettes per day ☐ Years smoked for ☐ Prev excess Y/N

**Family History** _____

**Medications and duration**

Aspirin _____
PPI _____
Statin _____
Anticoagulant ____
Steroid _____
Blockers _____

***Co-Morbidities***

*MI* ☐ *CCF* ☐
*PVD* ☐ *CVA* ☐
*Dementia* ☐ *COPD* ☐
*ConTissDis* ☐ *Ulcer* ☐
*Liver (mild)* ☐ *DM (OK)* ☐
*Hemiplegia* ☐ *CKD>3* ☐
*DM (damage)* ☐ *Tumour* ☐
*Leukaemia* ☐ *Lymph* ☐
*Mod/Sev Liver disease* ☐
*Metastatic tumour* ☐
*AIDS* ☐

*(Tick = Yes, Cross = No)*

**Measurements** _____ Kg _____ m (current)

_____ Kg loss over _____ Weeks

_____ Kg 4 weeks ago _____ Kg 1 year ago

Exercise tolerance (Current) 0-100m / 100-1000m / u/l
(4 weeks ago) 0-100m / 100-1000m / u/l
(1 year ago) 0-100m / 100-1000m / u/l

Timed up-and-go
Lap 1 (sit-to-stand) _____
Lap 2 (3m walk) _____
Lap 3 (turn) _____
Lap 4 (3m walk) _____
Total _____

PFTs
FEV1 (l/min)/%predicted _____ _____%
FVC (l/min)/%predicted _____ _____%
FEV1/FVC _____%_____%

Bloods
Hb _____
Albumin _____
eGFR _____
CRP _____
HbA1c _____
Urea _____
Creatinine _____

**Tumour**

Type Adenocarcinoma / Squamous Cell Carcinoma / Nil / Other _____

Site Oesophagus / Junction / Stomach / Pancreas / Nil / Other _____

**Staging**

| | | | | | |
|---|---|---|---|---|---|
| CT | Tumour_____ | PET | Tumour_____ | EUS | Tumour_____ |
| | Nodes _____ | | Nodes _____ | | Nodes _____ |
| | Mets _____ | | Mets _____ | | Mets _____ |

Diagnostic Laparoscopy Y/N

**Chemotherapy**

Neo-adjuvant / Palliative / Curative / None

2xCF / 3xECX / 4xECX / 3xEOX / 6xEOC / 6xECF

Other _____

Not Started / Completed / Incomplete

Complications _____

Patient choice _____

**Post-Chemo staging**

Complete Response ☐

Partial Response ☐

Stable Disease ☐

Progressive disease – still curative ☐

Progressive disease – Palliative ☐

**Surgery**

Date _____ Operator CD / RJES / GWC / AMXP / PJXL / Other _____

Abdominal – Open / Laparoscopic / Laparoscopic converted to open

Thoracic – Open / Thoracoscopic / Thoracoscopic converted to open

Thoracoabdominal / Trans-hiatal / Thoracoscopic

Whipples / Central liver resection for cholangiocarcinoma

Vascular – TAAA / AAA / Aorto-femoral bypass for occlusive disease / Mesenteric bypass

Other – Live donor nephrectomy / Incisional hernia / Other _____

**Pathology**

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| Final staging | Tumour_____ | Nodes _____ | Mets _____ | | | | R0 / R1 |
| Nodes | Number__/___ | Above diaphragm | Y/N | Below diaphragm | Y/N | | |
| Lymphovascular invasion | Y/N | Perineural invasion | Y/N | | | | |
| Extramural vascular invasion | Y/N | Circumferential margin | +ve / -ve | | | | |
| Proximal margin | +ve / -ve | Distal margin | +ve / -ve | | | | |

PG-SGA

**1. Weight**

In summary of my current and recent weight:

I currently weigh about _____ Kg  I am currently about _____ cm tall

1 month ago I weighed _____ Kg  6 months ago I weighed _____ Kg

In the last 2 weeks my weight has  Decreased  Not changed  Increased

**2. Food Intake**

As compared to my normal diet, I would rate my food intake during the past month as:

☐ Unchanged ☐ Increased ☐ Decreased

I am now taking

☐ *Normal food,* but less than a normal amount

☐ Little solid food ☐ Only liquids

☐ Only nutritional supplements ☐ Very little of anything

☐ Only tube feedings or nutrition by vein

**3. Symptoms**

I have had the following problems that have kept me from eating enough during the past 3 weeks:

☐ No problems eating

☐ No appetite, just didn't feel like eating ☐ Vomiting

☐ Nausea ☐ Diarrhoea

☐ Constipation ☐ Dry mouth

☐ Mouth sores ☐ Smells bother me

☐ Things taste funny or have no taste ☐ Feel full quickly

☐ Problems with swallowing ☐ Fatigue

☐ Pain – where? _____ ☐ Other _____

**4. Activities and functions**

Over the past month, I would generally rate my activity as:

☐ Normal with no limitations

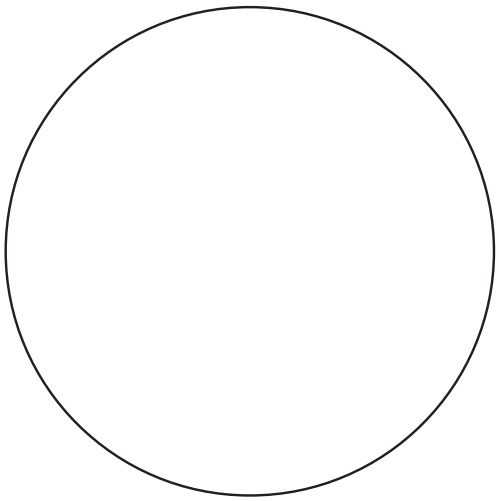
☐ Not my normal self, but able to be up and about with fairly normal activities

☐ Not feeling up to most things, but in bed or chair less than half the day

☐ Able to do little activity, and spend over half the day in bed or chair

☐ Pretty much bed ridden, rarely out of bed

Please imagine this pre-drawn circle is a clock. I would like you to place the numbers in the correct position and then place the hands to indicate a time of “ten after eleven”.



| | | | |
|---|---|---|---|
| In the past year, how many times have you been admitted to hospital? | 0 | 1-2 | Over 2 |
| In general, how would you describe your health? | Excellent / very good / good | Fair | Poor |
| With how many of the following activities do you require help?<br>Meal preparation / shopping / transportation / telephone / housekeeping / laundry / managing money / taking medications | 0-1 | 2-4 | 5-8 |
| When you need help, can you count on someone who is willing and able to meet your needs? | Always | Sometimes | Never |
| Do you use 5 or more different prescription medications on a regular basis? | No | Yes | |
| At times, do you forget to take your prescription medications? | No | Yes | |
| Have you recently lost weight such that your clothing has become looser? | No | Yes | |
| Do you often feel sad or depressed? | No | Yes | |
| Do you have a problem with losing control of urine when you don’t want to? | No | Yes | |
| Two weeks ago, were you able to: | | | |
| (1) Do heavy work around the house like washing windows, walls, or floors without help? | Yes | No | |
| (2) Walk up and down stairs to the second floor without help? | Yes | No | |
| (3) Walk 1km without help? | Yes | No | |


# Health Questionnaire

## English version for the UK

***(Validated for Ireland)***

By placing a tick in one box in each group below, please indicate which statements best describe your own health state today.

**Mobility**

I have no problems in walking about ❑

I have some problems in walking about ❑

I am confined to bed ❑

**Self-Care**

I have no problems with self-care ❑

I have some problems washing or dressing myself ❑

I am unable to wash or dress myself ❑

**Usual Activities** *(e.g. work, study, housework, family or leisure activities)*

I have no problems with performing my usual activities ❑

I have some problems with performing my usual activities ❑

I am unable to perform my usual activities ❑

**Pain / Discomfort**

I have no pain or discomfort ❑

I have moderate pain or discomfort ❑

I have extreme pain or discomfort ❑

**Anxiety / Depression**

I am not anxious or depressed ❑

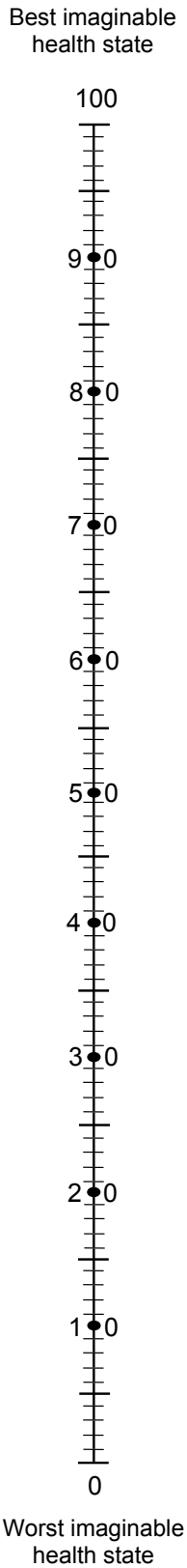
I am moderately anxious or depressed ❑

I am extremely anxious or depressed ❑

To help people say how good or bad a health state is, we have drawn a scale (rather like a thermometer) on which the best state you can imagine is marked 100 and the worst state you can imagine is marked 0.

We would like you to indicate on this scale how good or bad your own health is today, in your opinion. Please do this by drawing a line from the box below to whichever point on the scale indicates how good or bad your health state is today.

**Your own health state today**



# EORTC QLQ-C30 (version 3)

We are interested in some things about you and your health. Please answer all of the questions yourself by circling the number that best applies to you. There are no "right" or "wrong" answers. The information that you provide will remain strictly confidential.

Please fill in your initials: _____

Your birthdate (Day, Month, Year): _____

Today's date (Day, Month, Year): 31 _____

---

| | | Not at All | A Little | Quite a Bit | Very Much |
|---|---|---|---|---|---|
| 1. | Do you have any trouble doing strenuous activities, like carrying a heavy shopping bag or a suitcase? | 1 | 2 | 3 | 4 |
| 2. | Do you have any trouble taking a long walk? | 1 | 2 | 3 | 4 |
| 3. | Do you have any trouble taking a short walk outside of the house? | 1 | 2 | 3 | 4 |
| 4. | Do you need to stay in bed or a chair during the day? | 1 | 2 | 3 | 4 |
| 5. | Do you need help with eating, dressing, washing yourself or using the toilet? | 1 | 2 | 3 | 4 |

## During the past week:

| | | Not at All | A Little | Quite a Bit | Very Much |
|---|---|---|---|---|---|
| 6. | Were you limited in doing either your work or other daily activities? | 1 | 2 | 3 | 4 |
| 7. | Were you limited in pursuing your hobbies or other leisure time activities? | 1 | 2 | 3 | 4 |
| 8. | Were you short of breath? | 1 | 2 | 3 | 4 |
| 9. | Have you had pain? | 1 | 2 | 3 | 4 |
| 10. | Did you need to rest? | 1 | 2 | 3 | 4 |
| 11. | Have you had trouble sleeping? | 1 | 2 | 3 | 4 |
| 12. | Have you felt weak? | 1 | 2 | 3 | 4 |
| 13. | Have you lacked appetite? | 1 | 2 | 3 | 4 |
| 14. | Have you felt nauseated? | 1 | 2 | 3 | 4 |
| 15. | Have you vomited? | 1 | 2 | 3 | 4 |
| 16. | Have you been constipated? | 1 | 2 | 3 | 4 |

Please go on to the next page

| **During the past week:** | **Not at All** | **A Little** | **Quite a Bit** | **Very Much** |
|---|---|---|---|---|
| 17. Have you had diarrhea? | 1 | 2 | 3 | 4 |
| 18. Were you tired? | 1 | 2 | 3 | 4 |
| 19. Did pain interfere with your daily activities? | 1 | 2 | 3 | 4 |
| 20. Have you had difficulty in concentrating on things, like reading a newspaper or watching television? | 1 | 2 | 3 | 4 |
| 21. Did you feel tense? | 1 | 2 | 3 | 4 |
| 22. Did you worry? | 1 | 2 | 3 | 4 |
| 23. Did you feel irritable? | 1 | 2 | 3 | 4 |
| 24. Did you feel depressed? | 1 | 2 | 3 | 4 |
| 25. Have you had difficulty remembering things? | 1 | 2 | 3 | 4 |
| 26. Has your physical condition or medical treatment interfered with your family life? | 1 | 2 | 3 | 4 |
| 27. Has your physical condition or medical treatment interfered with your social activities? | 1 | 2 | 3 | 4 |
| 28. Has your physical condition or medical treatment caused you financial difficulties? | 1 | 2 | 3 | 4 |

## For the following questions please circle the number between 1 and 7 that best applies to you

29. How would you rate your overall health during the past week?

| 1 | 2 | 3 | 4 | 5 | 6 | 7 |
|---|---|---|---|---|---|---|
| Very poor | | | | | | Excellent |

30. How would you rate your overall quality of life during the past week?

| 1 | 2 | 3 | 4 | 5 | 6 | 7 |
|---|---|---|---|---|---|---|
| Very poor | | | | | | Excellent |

# EORTC QLQ – OG25

Patients sometimes report that they have the following symptoms or problems. Please indicate the extent to which you have experienced these symptoms or problems during the past week. Please answer by circling the number that best applies to you.

| **During the past week:** | **Not at all** | **A little** | **Quite a bit** | **Very much** |
|---|---|---|---|---|
| 31. Have you had problems eating solid foods? | 1 | 2 | 3 | 4 |
| 32. Have you had problems eating liquidised or soft foods? | 1 | 2 | 3 | 4 |
| 33. Have you had problems drinking liquids? | 1 | 2 | 3 | 4 |
| 34. Have you had trouble enjoying your meals? | 1 | 2 | 3 | 4 |
| 35. Have you felt full up too quickly after beginning to eat? | 1 | 2 | 3 | 4 |
| 36. Has it taken you a long time to complete your meals? | 1 | 2 | 3 | 4 |
| 37. Have you had difficulty eating? | 1 | 2 | 3 | 4 |
| 38. Have you had acid indigestion or heartburn? | 1 | 2 | 3 | 4 |
| 39. Has acid or bile coming into your mouth been a problem? | 1 | 2 | 3 | 4 |
| 40. Have you had discomfort when eating? | 1 | 2 | 3 | 4 |
| 41. Have you had pain when you eat? | 1 | 2 | 3 | 4 |
| 42. Have you had pain in your stomach area? | 1 | 2 | 3 | 4 |
| 43. Have you had discomfort in your stomach area? | 1 | 2 | 3 | 4 |
| 44. Have you been thinking about your illness? | 1 | 2 | 3 | 4 |
| 45. Have you worried about your health in the future? | 1 | 2 | 3 | 4 |
| 46. Have you had trouble with eating in front of other people? | 1 | 2 | 3 | 4 |
| 47. Have you had a dry mouth? | 1 | 2 | 3 | 4 |
| 48. Have you had problems with your sense of taste? | 1 | 2 | 3 | 4 |
| 49. Have you felt physically less attractive as a result of your disease or treatment? | 1 | 2 | 3 | 4 |

Please go on to the next page

| During the past week: | Not at all | A little | Quite a bit | Very much |
|---|---|---|---|---|
| 50. Have you had difficulty swallowing your saliva? | 1 | 2 | 3 | 4 |
| 51. Have you choked when swallowing? | 1 | 2 | 3 | 4 |
| 52. Have you coughed? | 1 | 2 | 3 | 4 |
| 53. Have you had difficulty talking? | 1 | 2 | 3 | 4 |
| 54. Have you worried about your weight being too low? | 1 | 2 | 3 | 4 |
| 55. Answer this question only if you lost any hair:<br>If so, were you upset by the loss of your hair? | 1 | 2 | 3 | 4 |

# References

[1] Katz AM, Katz PB. DISEASE OF THE HEART IN THE WORKS OF HIPPOCRATES. Br Heart J 1962;24:257–64.

[2] Dewys WD, Begg C, Lavin PT, Band PR, Bennett JM, Bertino JR, et al. Prognostic effect of weight loss prior to chemotherapy in cancer patients. Eastern cooperative oncology group. Am J Med 1980;69:491–7.

[3] Wallengren O, Lundholm K, Bosaeus I. Diagnostic criteria of cancer cachexia: Relation to quality of life, exercise capacity and survival in unselected palliative care patients. Support Care Cancer 2013;21:1569–77. https://doi.org/10.1007/s00520-012-1697-z.

[4] Oken MM, Creech RH, Tormey DC, Horton J, Davis TE, McFadden ET, et al. Toxicity and response criteria of the eastern cooperative oncology group. Am J Clin Oncol 1982;5:649–55.

[5] Osler W. The principles and practice of medicine, designed for the use of practitioners and students of medicine. New York: D. Appleton and Company; 1901.

[6] Taylor HC. Cancer; its study and prevention, Philadelphia and New York: Lea and Febiger; 1915.

[7] Baumgartner RN, Koehler KM, Gallagher D, Romero L, Heymsfield SB, Ross RR, et al. Epidemiology of sarcopenia among the elderly in new mexico. American Journal of Epidemiology 1998;147:755–63.

[8] Argilés JM, López-Soriano FJ, Toledo M, Betancourt A, Serpe R, Busquets S. The cachexia score (CASCO): A new tool for staging cachectic cancer patients. J Cachexia Sarcopenia Muscle 2011;2:87–93. https://doi.org/10.1007/s13539-011-0027-5.

[9] Dahele M, Fearon K. Research methodology: Cancer cachexia syndrome. Palliative Medicine 2004;18:409–17. https://doi.org/10.1191/0269216304pm906ra.

[10] Bennani-Baiti N, Walsh D. What is cancer anorexia-cachexia syndrome? A historical perspective. J R

Coll Physicians Edinb 2009:6.

[11] Fox KM, Brooks JM, Gandra SR, Markus R, Chiou C-F. Estimation of cachexia among cancer patients based on four definitions. Journal of Oncology 2009;2009:1–7. https://doi.org/10.1155/2009/693458.

[12] Fearon K, Strasser F, Anker SD, Bosaeus I, Bruera E, Fainsinger RL, et al. Definition and classification of cancer cachexia: An international consensus. Lancet Oncol 2011;12:489–95. https://doi.org/10.1016/S1470-2045(10)70218-7.

[13] Haehling S von, Anker MS, Anker SD. Prevalence and clinical impact of cachexia in chronic illness in europe, usa, and japan: Facts and numbers update 2016. Journal of Cachexia, Sarcopenia and Muscle 2016;7:507–9. https://doi.org/10.1002/jcsm.12167.

[14] Blum D, Stene GB, Solheim TS, Fayers P, Hjermstad MJ, Baracos VE, et al. Validation of the consensus-definition for cancer cachexia and evaluation of a classification model—a study based on data from an international multicentre project (EPCRC-CSA). Ann Oncol 2014;25:1635–42. https://doi.org/10.1093/annonc/mdu086.

[15] Vanhoutte G, Wiel M van de, Wouters K, Sels M, Bartolomeeussen L, Keersmaecker SD, et al. Cachexia in cancer: What is in the definition? BMJ Open Gastroenterology 2016;3:e000097. https://doi.org/10.1136/bmjgast-2016-000097.

[16] Evans WJ, Morley JE, Argilés J, Bales C, Baracos V, Guttridge D, et al. Cachexia: A new definition. Clinical Nutrition 2008;27:793–9. https://doi.org/10.1016/j.clnu.2008.06.013.

[17] Mourtzakis M, Prado CM, Lieffers JR, Reiman T, McCargar LJ, Baracos VE. A practical and precise approach to quantification of body composition in cancer patients using computed tomography images acquired during routine care. Applied Physiology, Nutrition, and Metabolism 2008;33:997–1006. https://doi.org/10.1139/H08-075.

[18] Martin L, Birdsell L, MacDonald N, Reiman T, Clandinin MT, McCargar LJ, et al. Cancer cachexia in the age of obesity: Skeletal muscle depletion is a powerful prognostic factor, independent of body mass index. JCO 2013;31:1539–47. https://doi.org/10.1200/JCO.2012.45.2722.

[19] Martin L, Senesse P, Gioulbasanis I, Antoun S, Bozzetti F, Deans C, et al. Diagnostic criteria for the classification of cancer-associated weight loss. JCO 2015;33:90–9. https://doi.org/10.1200/JCO.2014.56.1894.

[20] Vagnildhaug OM, Blum D, Wilcock A, Fayers P, Strasser F, Baracos VE, et al. The applicability of a weight loss grading system in cancer cachexia: A longitudinal analysis. Journal of Cachexia, Sarcopenia and Muscle 2017;8:789–97. https://doi.org/10.1002/jcsm.12220.

[21] Elia PM. Nutritional screening of adults: A multidisciplinary responsibility 2003:138.

[22] Bauer J, Capra S, Ferguson M. Use of the scored patient-generated subjective global assessment (PG-SGA) as a nutrition assessment tool in patients with cancer. European Journal of Clinical Nutrition 2002;56:779–85. https://doi.org/10.1038/sj.ejcn.1601412.

[23] MacDonald N. Terminology in cancer cachexia: Importance and status. Current Opinion in Clinical Nutrition and Metabolic Care 2012;15:220–5. https://doi.org/10.1097/MCO.0b013e328352a895.

[24] Fearon KCH, Von Meyenfeldt MF, Moses AGW, Van Geenen R, Roy A, Gouma DJ, et al. Effect of a protein and energy dense n-3 fatty acid enriched oral supplement on loss of weight and lean tissue in cancer cachexia: A randomised double blind trial. Gut 2003;52:1479–86.

[25] Temel JS, Abernethy AP, Currow DC, Friend J, Duus EM, Yan Y, et al. Anamorelin in patients with non-small-cell lung cancer and cachexia (ROMANA 1 and ROMANA 2): Results from two randomised, double-blind, phase 3 trials. The Lancet Oncology 2016;17:519–31. https://doi.org/10.1016/S1470-2045(15)00558-6.

[26] Currow D, Temel JS, Abernethy A, Milanowski J, Friend J, Fearon KC. ROMANA 3: A phase 3 safety extension study of anamorelin in advanced non-small-cell lung cancer (NSCLC) patients with cachexia. Ann Oncol 2017;28:1949–56. https://doi.org/10.1093/annonc/mdx192.

[27] Crawford J, Johnston MA, Taylor RP, Dalton JT, Steiner MS. Enobosarm and lean body mass in patients with non-small cell lung cancer. J Clin Oncol 2014;32:5s.

[28] Deans DAC, Wigmore SJ, Gilmour H, Paterson-Brown S, Ross JA, Fearon KCH. Elevated tumour interleukin-1beta is associated with systemic inflammation: A marker of reduced survival in gastro-oesophageal cancer. British Journal of Cancer 2006;95:1568–75. https://doi.org/10.1038/sj.bjc.6603446.

[29] Dolan RD, Lim J, McSorley ST, Horgan PG, McMillan DC. The role of the systemic inflammatory response in predicting outcomes in patients with operable cancer: Systematic review and meta-analysis. Sci Rep 2017;7:16717. https://doi.org/10.1038/s41598-017-16955-5.

[30] Dolan RD, McSorley ST, Horgan PG, Laird B, McMillan DC. The role of the systemic inflammatory

response in predicting outcomes in patients with advanced inoperable cancer: Systematic review and meta-analysis. Critical Reviews in Oncology/Hematology 2017;116:134–46. https://doi.org/10.1016/j.critrevonc.2017.06.002.

[31] McSorley ST, Roxburgh CSD, Horgan PG, McMillan DC. The relationship between cardiopulmonary exercise test variables, the systemic inflammatory response, and complications following surgery for colorectal cancer. Perioper Med (Lond) 2018;7:11. https://doi.org/10.1186/s13741-018-0093-8.

[32] Roxburgh CSD, McMillan DC. Cancer and systemic inflammation: Treat the tumour and treat the host. Br J Cancer 2014;110:1409–12. https://doi.org/10.1038/bjc.2014.90.

[33] Morhij R, Mahendra A, Jane M, McMillan DC. The modified Glasgow prognostic score in patients undergoing surgery for bone and soft tissue sarcoma. Journal of Plastic, Reconstructive & Aesthetic Surgery 2017;70:618–24. https://doi.org/10.1016/j.bjps.2017.01.016.

[34] Guthrie GJK, Charles KA, Roxburgh CSD, Horgan PG, McMillan DC, Clarke SJ. The systemic inflammation-based neutrophil–lymphocyte ratio: Experience in patients with cancer. Critical Reviews in Oncology/Hematology 2013;88:218–30. https://doi.org/10.1016/j.critrevonc.2013.03.010.

[35] Desborough JP. The stress response to trauma and surgery. Br J Anaesth 2000;85:109–17. https://doi.org/10.1093/bja/85.1.109.

[36] Forrest LM, McMillan DC, McArdle CS, Angerson WJ, Dunlop DJ. Evaluation of cumulative prognostic scores based on the systemic inflammatory response in patients with inoperable non-small-cell lung cancer. Br J Cancer 2003;89:1028–30. https://doi.org/10.1038/sj.bjc.6601242.

[37] McMillan DC, Crozier JEM, Canna K, Angerson WJ, McArdle CS. Evaluation of an inflammation-based prognostic score (gps) in patients undergoing resection for colon and rectal cancer. International Journal of Colorectal Disease 2007;22:881. https://doi.org/10.1007/s00384-006-0259-6.

[38] McMillan DC, Canna K, McArdle CS. Systemic inflammatory response predicts survival following curative resection of colorectal cancer. British Journal of Surgery 2003;90:215–9. https://doi.org/10.1002/bjs.4038.

[39] Roxburgh CSD, McMillan DC. Cancer and systemic inflammation: Treat the tumour and treat the host. British Journal of Cancer 2014;110:1409–12. https://doi.org/10.1038/bjc.2014.90.

[40] Dolan RD, Almasaudi AS, Dieu LB, Horgan PG, McSorley ST, McMillan DC. The relationship between computed tomography-derived body composition, systemic inflammatory response, and survival in patients

undergoing surgery for colorectal cancer. Journal of Cachexia, Sarcopenia and Muscle 2019;10:111–22. https://doi.org/10.1002/jcsm.12357.

[41] Xiao J, Caan BJ, Weltzien E, Cespedes Feliciano EM, Kroenke CH, Meyerhardt JA, et al. Associations of pre-existing co-morbidities with skeletal muscle mass and radiodensity in patients with non-metastatic colorectal cancer. Journal of Cachexia, Sarcopenia and Muscle 2018;9:654–63. https://doi.org/10.1002/jcsm.12301.

[42] Badakhshi H, Kaul D, Zhao K-L. Association between the inflammatory biomarker, C-reactive protein, and the response to radiochemotherapy in patients with esophageal cancer. Molecular and Clinical Oncology 2016;4:643–7. https://doi.org/10.3892/mco.2016.753.

[43] Park JK, Kim JJ, Moon SW. C-reactive protein for the early prediction of anastomotic leak after esophagectomy in both neoadjuvant and non-neoadjuvant therapy case: A propensity score matching analysis. J Thorac Dis 2017;9:3693–702. https://doi.org/10.21037/jtd.2017.08.125.

[44] Hyman N, Manchester TL, Osler T, Burns B, Cataldo PA. Anastomotic leaks after intestinal anastomosis: It's later than you think. Ann Surg 2007;245:254–8. https://doi.org/10.1097/01.sla.0000225083.27182.85.

[45] Emery PW, Edwards RH, Rennie MJ, Souhami RL, Halliday D. Protein synthesis in muscle measured in vivo in cachectic patients with cancer. Br Med J (Clin Res Ed) 1984;289:584–6. https://doi.org/10.1136/bmj.289.6445.584.

[46] Williams JP, Phillips BE, Smith K, Atherton PJ, Rankin D, Selby AL, et al. Effect of tumor burden and subsequent surgical resection on skeletal muscle mass and protein turnover in colorectal cancer patients. Am J Clin Nutr 2012;96:1064–70. https://doi.org/10.3945/ajcn.112.045708.

[47] Dijk DP van, Poll MC van de, Moses AG, Preston T, Olde Damink SW, Rensen SS, et al. Effects of oral meal feeding on whole body protein breakdown and protein synthesis in cachectic pancreatic cancer patients. J Cachexia Sarcopenia Muscle 2015;6:212–21. https://doi.org/10.1002/jcsm.12029.

[48] Deutz NE, Safar A, Schutzler S, Memelink R, Ferrando A, Spencer H, et al. Muscle protein synthesis in cancer patients can be stimulated with a specially formulated medical food. Clin Nutr 2011;30:759–68. https://doi.org/10.1016/j.clnu.2011.05.008.

[49] MacDonald AJ, Small AC, Greig CA, Husi H, Ross JA, Stephens NA, et al. A novel oral tracer procedure for measurement of habitual myofibrillar protein synthesis. Rapid Commun Mass Spectrom 2013;27:1769–77. https://doi.org/10.1002/rcm.6622.

[50] MacDonald AJ, Johns N, Stephens N, Greig C, Ross JA, Small AC, et al. Habitual myofibrillar protein synthesis is normal in patients with upper GI cancer cachexia. Clin Cancer Res 2015;21:1734–40. https://doi.org/10.1158/1078-0432.CCR-14-2004.

[51] Brosnan JT. Interorgan amino acid transport and its regulation. J Nutr 2003;133:2068S–72S. https://doi.org/10.1093/jn/133.6.2068S.

[52] Souba WW. Review: Interorgan Ammonia Metabolism in Health and Disease: A Surgeon's View. Journal of Parenteral and Enteral Nutrition 1987;11:569–79. https://doi.org/10.1177/0148607187011006569.

[53] Pisters PW, Brennan MF. Amino acid metabolism in human cancer cachexia. Annu Rev Nutr 1990;10:107–32. https://doi.org/10.1146/annurev.nu.10.070190.000543.

[54] Argilés JM, López-Soriano FJ, Busquets S. Mediators of cachexia in cancer patients. Nutrition 2019;66:11–5. https://doi.org/10.1016/j.nut.2019.03.012.

[55] Dworzak F, Ferrari P, Gavazzi C, Maiorana C, Bozzetti F. Effects of cachexia due to cancer on whole body and skeletal muscle protein turnover. Cancer 1998;82:42–8. https://doi.org/10.1002/(SICI)1097-0142(19980101)82:1<42::AID-CN

[56] Penna F, Ballarò R, Beltrà M, De Lucia S, García Castillo L, Costelli P. The Skeletal Muscle as an Active Player Against Cancer Cachexia. Front Physiol 2019;10:41. https://doi.org/10.3389/fphys.2019.00041.

[57] Douglas E, McMillan DC. Towards a simple objective framework for the investigation and treatment of cancer cachexia: The Glasgow Prognostic Score. Cancer Treatment Reviews 2014;40:685–91. https://doi.org/10.1016/j.ctrv.2013.11.007.

[58] Llop-Talaveron J, Badia-Tahull MB, Leiva-Badosa E. An inflammation-based prognostic score, the C-reactive protein/albumin ratio predicts the morbidity and mortality of patients on parenteral nutrition. Clinical Nutrition 2018;37:1575–83. https://doi.org/10.1016/j.clnu.2017.08.013.

[59] Aubrey J, Esfandiari N, Baracos VE, Buteau FA, Frenette J, Putman CT, et al. Measurement of skeletal muscle radiation attenuation and basis of its biological variation. Acta Physiol (Oxf) 2014;210:489–97. https://doi.org/10.1111/apha.12224.

[60] Rollins KE, Javanmard-Emamghissi H, Awwad A, Macdonald IA, Fearon KCH, Lobo DN. Body composition measurement using computed tomography: Does the phase of the scan matter? Nutrition 2017;41:37–44. https://doi.org/10.1016/j.nut.2017.02.011.

[61] Vugt JLA van, Coebergh van den Braak RRJ, Schippers HJW, Veen KM, Levolger S, Bruin RWF de, et

al. Contrast-enhancement influences skeletal muscle density, but not skeletal muscle mass, measurements on computed tomography. Clinical Nutrition 2017. https://doi.org/10.1016/j.clnu.2017.07.007.

[62] Dickerson JWT, Widdowson EM. Chemical changes in skeletal muscle during development. Biochemical Journal 1960;74:247–57. https://doi.org/10.1042/bj0740247.

[63] Janney N. The protein content of muscle. J Biol Chem 1916;25:185.

[64] Lentner C, editor. Geigy scientific tables: Units of measurement, body fluids, composition of the body, nutrition v. 1. Novartis; 1981.

[65] Prado CM, Lieffers JR, McCargar LJ, Reiman T, Sawyer MB, Martin L, et al. Prevalence and clinical implications of sarcopenic obesity in patients with solid tumours of the respiratory and gastrointestinal tracts: A population-based study. The Lancet Oncology 2008;9:629–35.

[66] Johns N, Hatakeyama S, Stephens NA, Degen M, Degen S, Frieauff W, et al. Clinical classification of cancer cachexia: Phenotypic correlates in human skeletal muscle. PLoS ONE 2014;9:e83618. https://doi.org/10.1371/journal.pone.0083618.

[67] Cohen S, Brault JJ, Gygi SP, Glass DJ, Valenzuela DM, Gartner C, et al. During muscle atrophy, thick, but not thin, filament components are degraded by murf1-dependent ubiquitylation. The Journal of Cell Biology 2009;185:1083–95. https://doi.org/10.1083/jcb.200901052.

[68] Rollins KE, Tewari N, Ackner A, Awwad A, Madhusudan S, Macdonald IA, et al. The impact of sarcopenia and myosteatosis on outcomes of unresectable pancreatic cancer or distal cholangiocarcinoma. Clin Nutr 2016;35:1103–9. https://doi.org/10.1016/j.clnu.2015.08.005.

[69] Stephens NA, Skipworth RJE, MacDonald AJ, Greig CA, Ross JA, Fearon KCH. Intramyocellular lipid droplets increase with progression of cachexia in cancer patients. Journal of Cachexia, Sarcopenia and Muscle 2011;2:111–7. https://doi.org/10.1007/s13539-011-0030-x.

[70] Barber MD, Ross JA, Fearon KCH. Changes in nutritional, functional, and inflammatory markers in advanced pancreatic cancer. Nutrition and Cancer 1999;35:106–10. https://doi.org/10.1207/S15327914NC352/_2.

[71] Falconer JS, Fearon KCH, Ross JA, Elton R, Wigmore SJ, James OJ, et al. Acute-phase protein response and survival duration of patients with pancreatic cancer. Cancer 1995;75:2077–82. https://doi.org/10.1002/1097-0142(19950415)75:8<2077::AID-CNCR2820750808>3.0.CO;2-9.

[72] Tisdale MJ. Catabolic mediators of cancer cachexia: Current Opinion in Supportive and Palliative Care 2008;2:256–61. https://doi.org/10.1097/SPC.0b013e328319d7fa.

[73] Kir S, White JP, Kleiner S, Kazak L, Cohen P, Baracos VE, et al. Tumour-derived PTH-related protein triggers adipose tissue browning and cancer cachexia. Nature 2014;513:100–4. https://doi.org/10.1038/nature13528.

[74] Deans C, Wigmore S, Paterson-Brown S, Black J, Ross J, Fearon KCH. Serum parathyroid hormone-related peptide is associated with systemic inflammation and adverse prognosis in gastroesophageal carcinoma. Cancer 2005;103:1810–8. https://doi.org/10.1002/cncr.20972.

[75] Kir S, Komaba H, Garcia AP, Economopoulos KP, Liu W, Lanske B, et al. PTH/PTHrP receptor mediates cachexia in models of kidney failure and cancer. Cell Metabolism 2016;23:315–23. https://doi.org/10.1016/j.cmet.2015.11.003.

[76] Chicheportiche Y, Bourdon PR, Xu H, Hsu Y-M, Scott H, Hession C, et al. TWEAK, a new secreted ligand in the tumor necrosis factor family that weakly induces apoptosis. J Biol Chem 1997;272:32401–10. https://doi.org/10.1074/jbc.272.51.32401.

[77] Dogra C, Changotra H, Wedhas N, Qin X, Wergedal JE, Kumar A. TNF-related weak inducer of apoptosis (TWEAK) is a potent skeletal muscle-wasting cytokine. FASEB J 2007;21:1857–69. https://doi.org/10.1096/fj.06-7537com.

[78] Wiley SR, Cassiano L, Lofton T, Davis-Smith T, Winkles JA, Lindner V, et al. A novel TNF receptor family member binds TWEAK and is implicated in angiogenesis. Immunity 2001;15:837–46. https://doi.org/10.1016/S1074-7613(01)00232-1.

[79] Feng S-LY, Guo Y, Factor VM, Thorgeirsson SS, Bell DW, Testa JR, et al. The fn14 immediate-early response gene is induced during liver regeneration and highly expressed in both human and murine hepatocellular carcinomas. The American Journal of Pathology 2000;156:1253–61. https://doi.org/10.1016/S0002-9440(10)64996-6.

[80] Winkles JA. The TWEAK–fn14 cytokine–receptor axis: Discovery, biology and therapeutic targeting. Nat Rev Drug Discov 2008;7:411–25. https://doi.org/10.1038/nrd2488.

[81] Raue U, Jemiolo B, Yang Y, Trappe S. TWEAK-fn14 pathway activation after exercise in human skeletal muscle: Insights from two exercise modes and a time course investigation. Journal of Applied Physiology 2015;118:569–78. https://doi.org/10.1152/japplphysiol.00759.2014.

[82] Bhatnagar S, Mittal A, Gupta SK, Kumar A. TWEAK causes myotube atrophy through coordinated activation of ubiquitin-proteasome system, autophagy, and caspases. J Cell Physiol 2012;227:1042–51. https://doi.org/10.1002/jcp.22821.

[83] Johnston AJ, Murphy KT, Jenkinson L, Laine D, Emmrich K, Faou P, et al. Targeting of fn14 prevents cancer-induced cachexia and prolongs survival. Cell 2015;162:1365–78. https://doi.org/10.1016/j.cell.2015.08.031.

[84] Brink M, Wellen J, Delafontaine P. Angiotensin II causes weight loss and decreases circulating insulin-like growth factor i in rats through a pressor-independent mechanism. J Clin Invest 1996;97:2509–16.

[85] Adigun AQ, Ajayi AA. The effects of enalapril-digoxin-diuretic combination therapy on nutritional and anthropometric indices in chronic congestive heart failure: Preliminary findings in cardiac cachexia. Eur J Heart Fail 2001;3:359–63.

[86] Russell ST, Sanders PM, Tisdale MJ. Angiotensin II directly inhibits protein synthesis in murine myotubes. Cancer Letters 2006;231:290–4. https://doi.org/10.1016/j.canlet.2005.02.007.

[87] Sanders PM, Russell ST, Tisdale MJ. Angiotensin II directly induces muscle protein catabolism through the ubiquitin–proteasome proteolytic pathway and may play a role in cancer cachexia. Br J Cancer 2005;93:425–34. https://doi.org/10.1038/sj.bjc.6602725.

[88] Yoshida T, Tabony AM, Galvez S, Mitch WE, Higashi Y, Sukhanov S, et al. Molecular mechanisms and signaling pathways of angiotensin II-induced muscle wasting: Potential therapeutic targets for cardiac cachexia. Int J Biochem Cell Biol 2013;45:2322–32. https://doi.org/10.1016/j.biocel.2013.05.035.

[89] Penafuerte CA, Gagnon B, Sirois J, Murphy J, MacDonald N, Tremblay ML. Identification of neutrophil-derived proteases and angiotensin II as biomarkers of cancer cachexia. Br J Cancer 2016;114:680–7. https://doi.org/10.1038/bjc.2016.3.

[90] Johns N, Stretch C, Tan BH, Solheim TS, Sørhaug S, Stephens NA, et al. New genetic signatures associated with cancer cachexia as defined by low skeletal muscle index and weight loss. Journal of Cachexia, Sarcopenia and Muscle 2016:n/a–a. https://doi.org/10.1002/jcsm.12138.

[91] Anker SD, Negassa A, Coats AJ, Afzal R, Poole-Wilson PA, Cohn JN, et al. Prognostic importance of weight loss in chronic heart failure and the effect of treatment with angiotensin-converting-enzyme inhibitors: An observational study. The Lancet 2003;361:1077–83. https://doi.org/10.1016/S0140-6736(03)12892-9.

[92] Cruz-Jentoft AJ, Baeyens JP, Bauer JM, Boirie Y, Cederholm T, Landi F, et al. Sarcopenia: European consensus on definition and diagnosis report of the european working group on sarcopenia in older people. Age Ageing 2010;39:412–23. https://doi.org/10.1093/ageing/afq034.

[93] MacDonald AJ, Greig CA, Baracos V. The advantages and limitations of cross-sectional body composition analysis. Curr Opin Support Palliat Care 2011;5:342–9. https://doi.org/10.1097/SPC.0b013e32834c49eb.

[94] Gallagher D, Visser M, De Meersman RE, Sepúlveda D, Baumgartner RN, Pierson RN, et al. Appendicular skeletal muscle mass: Effects of age, gender, and ethnicity. J Appl Physiol 1997;83:229–39.

[95] Brooks RA. A quantitative theory of the hounsfield unit and its application to dual energy scanning. Journal of Computer Assisted Tomography 1977;1:487–93.

[96] Agha M, Agha R. The rising prevalence of obesity: Part a: Impact on public health. Int J Surg Oncol (N Y) 2017;2:e17. https://doi.org/10.1097/IJ9.0000000000000017.

[97] Carrara G, Pecorelli N, Cobelli FD, Cristel G, Damascelli A, Beretta L, et al. Preoperative sarcopenia determinants in pancreatic cancer patients. Clinical Nutrition 2017;36:1649–53. https://doi.org/https://doi.org/10.1016/j.clnu.2016.10.014.

[98] Villaseñor A, Ballard-Barbash R, Baumgartner K, Baumgartner R, Bernstein L, McTiernan A, et al. Prevalence and prognostic effect of sarcopenia in breast cancer survivors: The HEAL study. J Cancer Surviv 2012;6:398–406. https://doi.org/10.1007/s11764-012-0234-x.

[99] Sabel MS, Lee J, Cai S, Englesbe MJ, Holcombe S, Wang S. Sarcopenia as a prognostic factor among patients with stage III melanoma. Ann Surg Oncol 2011;18:3579–85. https://doi.org/10.1245/s10434-011-1976-9.

[100] Otten L, Stobäus N, Franz K, Genton L, Müller-Werdan U, Wirth R, et al. Impact of sarcopenia on 1-year mortality in older patients with cancer. Age Ageing 2019;48:413–8. https://doi.org/10.1093/ageing/afy212.

[101] Harimoto N, Shirabe K, Yamashita Y-I, Ikegami T, Yoshizumi T, Soejima Y, et al. Sarcopenia as a predictor of prognosis in patients following hepatectomy for hepatocellular carcinoma. BJS 2013;100:1523–30. https://doi.org/10.1002/bjs.9258.

[102] Cunha LP da, Silveira MN, Mendes MCS, Costa FO, Macedo LT, Siqueira NS de, et al. Sarcopenia as an independent prognostic factor in patients with metastatic colorectal cancer: A retrospective evaluation. Clinical Nutrition ESPEN 2019;32:107–12. https://doi.org/10.1016/j.clnesp.2019.04.004.

[103] Go S-I, Park MJ, Song H-N, Kim H-G, Kang MH, Lee HR, et al. Prognostic impact of sarcopenia in patients with diffuse large b-cell lymphoma treated with rituximab plus cyclophosphamide, doxorubicin, vincristine, and prednisone. Journal of Cachexia, Sarcopenia and Muscle 2016;7:567–76. https://doi.org/10.1002/jcsm.12115.

[104] Harada K, Ida S, Baba Y, Ishimoto T, Kosumi K, Tokunaga R, et al. Prognostic and clinical impact of sarcopenia in esophageal squamous cell carcinoma. Dis Esophagus 2016;29:627–33. https://doi.org/10.1111/dote.12381.

[105] Lee SJ, Park YJ, Cartmell KB. Sarcopenia in cancer survivors is associated with increased cardiovascular disease risk. Support Care Cancer 2018;26:2313–21. https://doi.org/10.1007/s00520-018-4083-7.

[106] Grinsven J van, Vugt JLA van, Gharbharan A, Bollen TL, Besselink MG, Santvoort HC van, et al. The association of computed tomography-assessed body composition with mortality in patients with necrotizing pancreatitis. J Gastrointest Surg 2017;21:1000–8. https://doi.org/10.1007/s11605-016-3352-3.

[107] Dijk DPJ van, Bakens MJAM, Coolsen MME, Rensen SS, Dam RM van, Bours MJL, et al. Low skeletal muscle radiation attenuation and visceral adiposity are associated with overall survival and surgical site infections in patients with pancreatic cancer. Journal of Cachexia, Sarcopenia and Muscle 2017;8:317–26. https://doi.org/10.1002/jcsm.12155.

[108] Paula NS, Rodrigues CS, Chaves GV. Comparison of the prognostic value of different skeletal muscle radiodensity parameters in endometrial cancer. European Journal of Clinical Nutrition 2018:1. https://doi.org/10.1038/s41430-018-0163-5.

[109] Williams GR, Deal AM, Muss HB, Weinberg MS, Sanoff HK, Nyrop KA, et al. Skeletal muscle measures and physical function in older adults with cancer: Sarcopenia or myopenia? Oncotarget 2017;8:33658–65. https://doi.org/10.18632/oncotarget.16866.

[110] Antoun S, Lanoy E, Iacovelli R, Albiges-Sauvin L, Loriot Y, Merad-Taoufik M, et al. Skeletal muscle density predicts prognosis in patients with metastatic renal cell carcinoma treated with targeted therapies. Cancer n.d.;119:3377–84. https://doi.org/10.1002/cncr.28218.

[111] Kazemi-Bajestani SMR, Mazurak VC, Baracos V. Computed tomography-defined muscle and fat wasting are associated with cancer clinical outcomes. Seminars in Cell & Developmental Biology 2016;54:2–10. https://doi.org/10.1016/j.semcdb.2015.09.001.

[112] Morsbach F, Zhang Y-H, Nowik P, Martin L, Lindqvist C, Svensson A, et al. Influence of tube potential

on CT body composition analysis. Nutrition 2018;53:9–13. https://doi.org/10.1016/j.nut.2017.12.016.

[113] Edmunds K, Gíslason M, Sigurðsson S, Guðnason V, Harris T, Carraro U, et al. Advanced quantitative methods in correlating sarcopenic muscle degeneration with lower extremity function biometrics and comorbidities. PLOS ONE 2018;13:e0193241. https://doi.org/10.1371/journal.pone.0193241.

[114] Safer U, Safer VB. Body composition analysis via single slice computed tomography analysis — the shortcomings. Journal of Geriatric Oncology 2016;7:53–4. https://doi.org/10.1016/j.jgo.2015.11.003.

[115] Cintosun U, Altun B, Tasci I. Sarcopenia is a condition with increasing importance in medical oncology. Oncologist 2016;21:e1. https://doi.org/10.1634/theoncologist.2015-0431.

[116] Safer U, Binay Safer V. Re: The impact of sarcopenia and myosteatosis on outcomes of unresectable pancreatic cancer or distal cholangiocarcinoma. Clinical Nutrition 2016;35:764. https://doi.org/10.1016/j.clnu.2016.03.007.

[117] Broughman JR, Williams GR, Muss HB. Response to "body composition analysis via single slice computed tomography analysis — the shortcomings". Journal of Geriatric Oncology 2016;7:55–6. https://doi.org/10.1016/j.jgo.2015.11.006.

[118] Torre-Vallejo M de la, Turcott J, Arrieta O, Baracos V. In reply. Oncologist 2016;21:e2. https://doi.org/10.1634/theoncologist.2015-0465.

[119] Mourtzakis M, Moisey L, Heyland D, Kozar R. Authors' response. Crit Care 2013;17:466.

[120] Rollins KE, Tewari N, Ackner A, Awwad A, Madhusudan S, Macdonald IA, et al. Reply, letter to the editor – the impact of sarcopenia and myosteatosis on outcomes of unresectable pancreatic cancer or distal cholangiocarcinoma. Clinical Nutrition 2016;35:765–6. https://doi.org/10.1016/j.clnu.2016.03.006.

[121] Baracos VE. Psoas as a sentinel muscle for sarcopenia: A flawed premise. J Cachexia Sarcopenia Muscle 2017;8:527–8. https://doi.org/10.1002/jcsm.12221.

[122] Rutten IJ, Ubachs J, Kruitwagen RF, Beets-Tan RG, Olde Damink SW, Van Gorp T. Psoas muscle area is not representative of total skeletal muscle area in the assessment of sarcopenia in ovarian cancer. Journal of Cachexia, Sarcopenia and Muscle 2017:n/a–a. https://doi.org/10.1002/jcsm.12180.

[123] Government of Canada SC. Immigration and ethnocultural diversity highlight tables - ethnic origin, both sexes, age (total), alberta, 2016 census – 25% sample data 2017. https://www12.statcan.gc.ca/census-recensement/2016/dp-pd/hlt-fst/imm/Table.cfm?Lang=E&T=31&Geo=48&SO=4D (accessed June

18, 2018).

[124] Cruz-Jentoft AJ, Baeyens JP, Bauer JM, Boirie Y, Cederholm T, Landi F, et al. Sarcopenia: European consensus on definition and diagnosis. Age Ageing 2010;39:412–23. https://doi.org/10.1093/ageing/afq034.

[125] Kwon H-J, Ha Y-C, Park H-M. The reference value of skeletal muscle mass index for defining the sarcopenia of women in korea. J Bone Metab 2015;22:71–5. https://doi.org/10.11005/jbm.2015.22.2.71.

[126] Fujiwara N, Nakagawa H, Kudo Y, Tateishi R, Taguri M, Watadani T, et al. Sarcopenia, intramuscular fat deposition, and visceral adiposity independently predict the outcomes of hepatocellular carcinoma. Journal of Hepatology 2015;63:131–40. https://doi.org/10.1016/j.jhep.2015.02.031.

[127] Tan BHL, Birdsell LA, Martin L, Baracos VE, Fearon KCH. Sarcopenia in an overweight or obese patient is an adverse prognostic factor in pancreatic cancer. Clin Cancer Res 2009;15:6973–9. https://doi.org/10.1158/1078-0432.CCR-09-1525.

[128] Ebadi M, Martin L, Ghosh S, Field CJ, Lehner R, Baracos VE, et al. Subcutaneous adiposity is an independent predictor of mortality in cancer patients. Br J Cancer 2017;117:148–55. https://doi.org/10.1038/bjc.2017.149.

[129] Montano-Loza AJ, Mazurak VC, Ebadi M, Meza-Junco J, Sawyer MB, Baracos VE, et al. Visceral adiposity increases risk for hepatocellular carcinoma in male patients with cirrhosis and recurrence after liver transplant. Hepatology 2018;67:914–23. https://doi.org/10.1002/hep.29578.

[130] Yip C, Dinkel C, Mahajan A, Siddique M, Cook GJR, Goh V. Imaging body composition in cancer patients: Visceral obesity, sarcopenia and sarcopenic obesity may impact on clinical outcome. Insights Imaging 2015;6:489–97. https://doi.org/10.1007/s13244-015-0414-0.

[131] Mersmann F, Bohm S, Schroll A, Boeth H, Duda G, Arampatzis A. Muscle shape consistency and muscle volume prediction of thigh muscles. Scand J Med Sci Sports 2015;25:e208–13. https://doi.org/10.1111/sms.12285.

[132] Willis TA, Hollingsworth KG, Coombs A, Sveen M-L, Andersen S, Stojkovic T, et al. Quantitative muscle MRI as an assessment tool for monitoring disease progression in LGMD2I: A multicentre longitudinal study. PLoS One 2013;8. https://doi.org/10.1371/journal.pone.0070993.

[133] Karlsson A, Rosander J, Romu T, Tallberg J, Grönqvist A, Borga M, et al. Automatic and quantitative assessment of regional muscle volume by multi-atlas segmentation using whole-body water–fat MRI. J Magn

Reson Imaging 2015;41:1558–69. https://doi.org/10.1002/jmri.24726.

[134] Baracos VE, Martin L, Korc M, Guttridge DC, Fearon KCH. Cancer-associated cachexia. Nature Reviews Disease Primers 2018;4:17105. https://doi.org/10.1038/nrdp.2017.105.

[135] Martin L, Gioulbasanis I, Senesse P, Baracos VE. Cancer-associated malnutrition and ct-defined sarcopenia and myosteatosis are endemic in overweight and obese patients. Journal of Parenteral and Enteral Nutrition n.d.;0. https://doi.org/10.1002/jpen.1597.

[136] O'Brien S, Kavanagh RG, Carey BW, Maher MM, O'Connor OJ, Andrews EJ. The impact of sarcopenia and myosteatosis on postoperative outcomes in patients with inflammatory bowel disease. European Radiology Experimental 2018;2:37. https://doi.org/10.1186/s41747-018-0072-3.

[137] Fearon KCH. Cancer cachexia and fat-muscle physiology. N Engl J Med 2011;365:565–7. https://doi.org/10.1056/NEJMcibr1106880.

[138] MacDonald A, Miller J, Ramage M, Greig C, Stephens N, Jacobi C, et al. Cross sectional imaging of truncal and quadriceps muscles relates to different functional outcomes in cancer. Clinical Nutrition 2018. https://doi.org/https://doi.org/10.1016/j.clnu.2018.12.023.

[139] Ferrioli E, Skipworth RJE, Stenseth J, Wall L, Greig CA, Fearon KC. Association between quality of life and directly monitored activity in patients with upper gastrointestinal (GI) cancer. In:. Abstracts of the 5 th cachexia conference, barcelona, spain, december 5-8, 2009, vol. 1, Journal of Cachexia, Sarcopenia; Muscle; 2010, p. 142.

[140] Skipworth RJE, Stene GB, Dahele M, Hendry PO, Small AC, Blum D, et al. Patient-focused endpoints in advanced cancer: Criterion-based validation of accelerometer-based activity monitoring. Clinical Nutrition 2011;30:812–21. https://doi.org/10.1016/j.clnu.2011.05.010.

[141] Lee SH, Kim SJ, Han Y, Ryu YJ, Lee JH, Chang JH. Hand grip strength and chronic obstructive pulmonary disease in korea: An analysis in KNHANES VI. Int J Chron Obstruct Pulmon Dis 2017;12:2313–21. https://doi.org/10.2147/COPD.S142621.

[142] Norman K, Stobäus N, Smoliner C, Zocher D, Scheufele R, Valentini L, et al. Determinants of hand grip strength, knee extension strength and functional status in cancer patients. Clinical Nutrition 2010;29:586–91. https://doi.org/10.1016/j.clnu.2010.02.007.

[143] Rolfson DB, Majumdar SR, Tsuyuki RT, Tahir A, Rockwood K. Validity and reliability of the edmonton

frail scale. Age Ageing 2006;35:526–9. https://doi.org/10.1093/ageing/afl041.

[144] Podsiadlo D, Richardson S. The timed "up & go": A test of basic functional mobility for frail elderly persons. Journal of the American Geriatrics Society 1991;39:142–8. https://doi.org/10.1111/j.1532-5415.1991.tb01616.x.

[145] Wall JC, Bell C, Campbell S, Davis J. The timed get-up-and-go test revisited: Measurement of the component tasks. Journal of Rehabilitation Research and Development 2000;37:109.

[146] Blagden SP, Charman SC, Sharples LD, Magee LRA, Gilligan D. Performance status score: Do patients and their oncologists agree? Br J Cancer 2003;89:1022–7. https://doi.org/10.1038/sj.bjc.6601231.

[147] Laird BJ, Fallon M, Hjermstad MJ, Tuck S, Kaasa S, Klepstad P, et al. Quality of life in patients with advanced cancer: Differential association with performance status and systemic inflammatory response. Journal of Clinical Oncology 2016;34:2769–75. https://doi.org/10.1200/JCO.2015.65.7742.

[148] Bergman H, Ferrucci L, Guralnik J, Hogan DB, Hummel S, Karunananthan S, et al. Frailty: An emerging research and clinical paradigm—issues and controversies. J Gerontol A Biol Sci Med Sci 2007;62:731–7.

[149] Hilmer SN, Perera V, Mitchell S, Murnion BP, Dent J, Bajorek B, et al. The assessment of frailty in older people in acute care. Australasian Journal on Ageing 2009;28:182–8. https://doi.org/10.1111/j.1741-6612.2009.00367.x.

[150] Post MW. Definitions of quality of life: What has happened and how to move on. Top Spinal Cord Inj Rehabil 2014;20:167–80. https://doi.org/10.1310/sci2003-167.

[151] Elkinton JR. Medicine and the quality of life. Annals of Internal Medicine 1966;64:711. https://doi.org/10.7326/0003-4819-64-3-711.

[152] Schulz KF, Altman DG, Moher D. CONSORT 2010 statement: Updated guidelines for reporting parallel group randomised trials. BMJ 2010;340:c332. https://doi.org/10.1136/bmj.c332.

[153] Calvert M, Blazeby J, Altman DG, Revicki DA, Moher D, Brundage MD, et al. Reporting of patient-reported outcomes in randomized trials: The CONSORT PRO extension. JAMA 2013;309:814–22. https://doi.org/10.1001/jama.2013.879.

[154] Bye A, Sjøblom B, Wentzel-Larsen T, Grønberg BH, Baracos VE, Hjermstad MJ, et al. Muscle mass and association to quality of life in non-small cell lung cancer patients. J Cachexia Sarcopenia Muscle 2017;8:759–67. https://doi.org/10.1002/jcsm.12206.

[155] Fayers P, Bottomley A, EORTC Quality of Life Group, Quality of Life Unit. Quality of life research within the EORTC-the EORTC QLQ-c30. European organisation for research and treatment of cancer. Eur J Cancer 2002;38 Suppl 4:S125–133.

[156] EuroQol Group. EuroQol–a new facility for the measurement of health-related quality of life. Health Policy 1990;16:199–208.

[157] Acharyya S, Butchbach ME, Sahenk Z, Wang H, Saji M, Carathers M, et al. Dystrophin glycoprotein complex dysfunction: A regulatory link between muscular dystrophy and cancer cachexia. Cancer Cell 2005;8:421–32. https://doi.org/10.1016/j.ccr.2005.10.004.

[158] Charlson ME, Pompei P, Ales KL, MacKenzie CR. A new method of classifying prognostic comorbidity in longitudinal studies: Development and validation. J Chronic Dis 1987;40:373–83.

[159] Grose D, Morrison DS, Devereux G, Jones R, Sharma D, Selby C, et al. Comorbidities in lung cancer: Prevalence, severity and links with socioeconomic status and treatment. Postgrad Med J 2014;90:305–10. https://doi.org/10.1136/postgradmedj-2013-132186.

[160] Grose D, Morrison DS, Devereux G, Jones R, Sharma D, Selby C, et al. The impact of comorbidity upon determinants of outcome in patients with lung cancer. Lung Cancer 2015;87:186–92. https://doi.org/10.1016/j.lungcan.2014.11.012.

[161] Knuf KM, Maani CV, Cummings AK. Clinical agreement in the american society of anesthesiologists physical status classification. Perioper Med (Lond) 2018;7. https://doi.org/10.1186/s13741-018-0094-7.

[162] Duncan R, Essat M, Jones G, Booth A, Buckley Woods H, Poku E, et al. Systematic review and qualitative evidence synthesis of patient-reported outcome measures for abdominal aortic aneurysm. BJS 2017;104:317–27. https://doi.org/10.1002/bjs.10407.

[163] Anota A, Savina M, Bascoul-Mollevi C, Bonnetain F. QoLR: An r package for the longitudinal analysis of health-related quality of life in oncology. Journal of Statistical Software, Articles 2017;77:1–30. https://doi.org/10.18637/jss.v077.i12.

[164] Heymsfield SB, Smith R, Aulet M, Bensen B, Lichtman S, Wang J, et al. Appendicular skeletal muscle mass: Measurement by dual-photon absorptiometry. Am J Clin Nutr 1990;52:214–8.

[165] Irving BA, Weltman JY, Brock DW, Davis CK, Gaesser GA, Weltman A. NIH ImageJ and slice-o-matic computed tomography imaging software to quantify soft tissue. Obesity 2007;15:370–6. https://doi.org/10.

1038/oby.2007.573.

[166] Richards CH, Roxburgh CSD, MacMillan MT, Isswiasi S, Robertson EG, Guthrie GK, et al. The relationships between body composition and the systemic inflammatory response in patients with primary operable colorectal cancer. PLOS ONE 2012;7:e41883. https://doi.org/10.1371/journal.pone.0041883.

[167] Rollins KE, Awwad A, Macdonald IA, Lobo DN. A comparison of two different software packages for analysis of body composition using computed tomography images. Nutrition 2019;57:92–6. https://doi.org/https://doi.org/10.1016/j.nut.2018.06.003.

[168] R Core Team. R: A language and environment for statistical computing. Vienna, Austria: R Foundation for Statistical Computing; 2016.

[169] Harrison EM. FinalFit. 2019.

[170] Hebbali A. Olsrr | Rsquared Academy. 2020.

[171] Aristotle, Ross WD, Smith JA. The works of Aristotle. Oxford: Clarendon Press; 1908.

[172] Aaronson N, Ahmedzai S, Bergman B, Bullinger M, Cull A, Duez N, et al. The european organization for research and treatment of cancer QLQ-c30 - a quality-of-life instrument for use in international clinical trials in oncology. Journal of the National Cancer Institute 1993;85:365–76.

[173] Hof AL. Scaling gait data to body size. Gait & Posture 1996;4:222–3. https://doi.org/10.1016/0966-6362(95)01057-2.

[174] Stansfield BW, Hillman SJ, Hazlewood ME, Lawson AA, Mann AM, Loudon IR, et al. Normalized speed, not age, characterizes ground reaction force patterns in 5-to 12-year-old children walking at self-selected speeds. J Pediatr Orthop n.d.;21:395–402.

[175] Lowell JA, Schifferdecker C, Driscoll DF, Benotti PN, Bistrian BR. Postoperative fluid overload: Not a benign problem. Crit Care Med 1990;18:728–33.

[176] Hunter JD, Doddi M. Sepsis and the heart. Br J Anaesth 2010;104:3–11. https://doi.org/10.1093/bja/aep339.

[177] Shankaran M, Czerwieniec G, Fessler C, Wong P-yA, Killion S, Turner SM, et al. Dilution of oral d3-creatine to measure creatine pool size and estimate skeletal muscle mass: Development of a correction algorithm. Journal of Cachexia, Sarcopenia and Muscle 2018;9:540–6. https://doi.org/10.1002/jcsm.12278.

[178] Cawthon PM, Orwoll ES, Peters KE, Ensrud KE, Cauley JA, Kado DM, et al. Strong Relation Between Muscle Mass Determined by D3-creatine Dilution, Physical Performance, and Incidence of Falls and Mobility Limitations in a Prospective Cohort of Older Men. The Journals of Gerontology: Series A 2018;74:844–52. https://doi.org/10.1093/gerona/gly129.